

ACTA RADIOLOGICA

FOUNDED IN 1971 BY GÖSTA FORSSELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK, FINLAND, NORWAY AND SWEDEN

ONCOLOGY

RADIATION THERAPY, PHYSICS AND BIOLOGY

EDITOR

ERIK LINDGREN

ADVISORY BOARD

Oncology Lars-Gunnar Larsson
Radiation physics Gunnar Hettinger
Radiation biology Bernhard Tribukait

ASSOCIATE EDITOR

LEIF RUDHE ULF BERGVALL

EDITORIAL BOARD

Denmark G Thomsen S Kaas
Finland P Virtama I R Holsti
Norway T Aakhus E Poppe
Sweden L-G Larsson G F Saltzman

Editorial

Laryngeal carcinoma—IV—Analysis of treatment results using the Cohen model

Complications from irradiation of carcinoma of the uterine cervix

Chromosome counts of ^{90}Sr induced osteosarcomas in mice—I—Transplanted tumour series

Effect of syngeneic bone marrow and thymus cell transplantation to ^{90}Sr irradiated mice

Simple experimentally derived algorithm for computer calculated dose rates associated with ^{137}Cs gynecologic insertions

Computation of dose distributions for radioactive seed implants

Effects of washing on phytohemagglutinin responsiveness of lymphocytes from irradiated patients

Procedures in External Radiation Therapy Dosimetry with Electron and Photon Beams with Maximum Energies between 1 and 50 MeV—Recommendations by the Nordic Association of Clinical Physics (NACP)

1

3 HJELM HANSEN M

13 ALERT J JIMENEZ J BELDARRAIN L MONTALVO²⁾ and ROCA C

17 BERGMAN H and NILSSON A

29 NILSSON A BIERKE P and BRONÉ KARLSSON A

37 WREDE D E and DAWALIBI H

41 ROSEN I I LANE R G and KELSEY C A

45 MATSUBARA S HORIUCHI J SHIBUYA H and SASAKI M S

55

ACTA RADIOLOGICA

FOUNDED IN 1971 BY GÖSTA FORSSELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK, FINLAND, SWEDEN AND NORWAY

ONCOLOGY

RADIATION THERAPY PHYSICS AND BIOLOGY

EDITOR
ERIK LINDGREN

ADVISORY BOARD
Oncology Lars-Gunnar Larsson
Radiation physics Gunnar Hettinger
Radiation biology Bernhard Tribukait

ASSOCIATE EDITORS
ULF RUDHE Ulf Hildebrandt

EDITORIAL BOARD
Denmark G. Thomsen & V. Vase
Finland P. Virtama & R. Hietala
Norway T. Aakhus & J. P. Foss
Sweden L.-G. Larsson & G. P. Sallén

Mitomycin C in advanced gallbladder carcinoma

Pentagastrin, calcium and whisky stimulated serum
calcitonin in medullary carcinoma of the thyroid

Neuron radiation therapy of parotid gland tumors

Effect of different radiation fractionation schedules on
metastases from an oesophageal carcinoma

Depth dose data for 4 MeV linear accelerators with
lead or uranium field flatteners

Dual photon absorptiometry in lumbar vertebrae—
Evaluation of the baseline error

Microdosimetry—I—Use of secondary electron
emission

Significance of quantum fluctuations in roentgen
imaging

Natural killer activity in peripheral lymphocyte
population following radiation therapy

Effect of different ^{90}Sr doses on the microscopic
structure of foetal mouse ovaries

Chromosome counts of ^{90}Sr induced osteosarcomas in
mice—II—Variation of the chromosome counts of
slow and fast growing tumours in hyper- and non-
hyperimmunized hosts

81 VON EYBEN F., HELLEKANT C., MAITAVI J.,
LIJNGQUIST U. and JONSSON K.

85 EMNERTSEN K., NIELSEN H. E., MØSEVOLDT J. &
HVID HANSEN H.

91 GERACI J. P.

99 MERCKE C., LAMMI L., NILSSON P., LÄNNIKÖ J.,
HÄKANSSON C. H. and HAMMAR C.

107 NAIR R. and WREDE D. E.

111 ROOS B. O., HANSSON T. H. and SÄLDGREN H.

115 FORSBERG B. J. and BURLIN T. E.

129 STRID K.-G.

139 BLOMGREN H., BARALE E., EDSMYR F., STRENDER
L. E., PETRINI B. and WASSERMAN J.

145 RÖNNBACK C.

153 BERGMAN H.

INSTRUCTIONS TO AUTHORS

ACTA RADIOLOGICA consists of two series: *Diagnosis* (red) including all medical imaging techniques and physiologic radiography and *Oncology* (blue) including radiation therapy, chemotherapy, nuclear therapy, radiation physics and radiation biology. Only original manuscripts will be considered for publication, implying that the material has not already been published nor is intended for publication elsewhere. Extensive articles may be published as Supplements for which special conditions apply. Decisions on publication are based on the opinions of at least two reviewers (whose names are not revealed to the authors). The manuscripts are subject to editorial revision. The right is reserved to introduce such changes as may be necessary to make contributions conform to the editorial standards. The revised manuscript is submitted to the authors before typesetting. The journal does not hold itself responsible for opinions or statements expressed by the authors. The copyright resides with *Acta Radiologica*.

Manuscripts. Only manuscripts written in English are accepted. They should be typed on one side of the paper only, with double spacing (at least 1 cm between the lines) and margins of at least 4 cm on the left side and at the top. The manuscript should be submitted in duplicate, and two complete sets of figures supplied. The name and address of the institution or hospital at which the work was carried out should be given on the title page. A summary, normally not exceeding 100 words, should be included. The author should also add an address to which correspondence can be directed, and retain a copy of the manuscript for reference. A request for reprints should include full name, address and post code.

Abbreviations should be spelled out when first used in the text. Uncommon abbreviations and clinical jargon should be avoided. Footnotes are not accepted. Tables should be numbered in Arabic numerals and given on separate sheets, each with a short descriptive title. Legends for illustrations should be given on a separate sheet.

Contributions should be brief and concise, carefully revised before submission to exclude unessential matter. Alterations in the proofs are expensive, and with the exception of typographic errors will be charged to the author.

Units and Mathematics. The International System of Units (SI) WHO 1975 should be used. A zero should always precede a decimal fraction (i.e. 0.123). Mathematical expressions should be set out clearly. Make a circled notation in the margin to explain possibly ambiguous characters such as 1 (numeral 1 and letter l), 0 (zero and letter O), \times (multiplication sign or letter x). In handwritten equations, mark clearly e and c, u, n and v, and letters in which capitals and lower case are nearly indistinguishable when handwritten, also primes and apostrophes. Use the solidus (/) for fractions and $\exp()$ when the exponent is complicated. In displayed formulae, the horizontal fraction line is preferable. Composite numbers should be expressed as $n \times m$

and not as $n \cdot m$. Equations should be numbered sequentially, Arabic numerals in parentheses on the right side of the page.

Illustrations. Glossy prints, unretouched and unmounted should be submitted. If the prints are not of a satisfactory standard, the author will be requested to submit the original film. Prints to be placed together should be of the same scale and of the same height to facilitate reproduction. The prints should have the same tonal relations as the original (for example, conventional angiographic films: white vessels on dark background; the reverse for subtraction films) with the patient's right to the reader's left; this is also valid for CT scans and other transverse images. Lettering and arrows are preferably marked lightly in pencil, either on the back or removable from the prints.

Diagrams and graphs should be clearly drawn in black (India) ink on a white background and submitted as glossy prints or the original art work. Lettering must be large enough to be legible after photographic reduction. Computer print-outs are usually satisfactory.

Colour drawings or photographs are accepted if the costs are defrayed by the author(s).

Each illustration must be provided with a suitable short legend, typewritten on a separate sheet and comprehensible without reference to the text. It must include explanations of all abbreviations and arrows used.

The figure number and the name of the first author must be marked lightly in pencil and the top indicated on the back of each illustration.

References. The references should be arranged in alphabetical order with name(s) of author(s) followed by initials, full title of the article, and name of the journal as abbreviated in *Index Medicus*. The volume number, year of publication in parentheses, and number of the first page of the article should follow. All authors of the article must be given in the reference list. Reference to books and monographs should indicate the author, the title and edition of the book, the name of the publishers, and the place and year of publication.

Examples

BOJSEN E and ZSIGMOND M. Selective angiography of bronchial and intercostal arteries. *Acta radiol. Diagnosis* 3 (1965) 513.
KEITH A. Human embryology and morphology. 6th edition. P 533. Arnold & Co. London 1948.

Reference in the running text to an article by one author (BOJSEN 1975), by two authors (BOJSEN & LIND 1975), by three or more authors (First author's name followed by et coll. (= co-workers) and not et al. (= and others)—(BOJSEN et coll. 1975)). The accuracy of reference data is the responsibility of the author.

One hundred reprints of each article are supplied free of charge; additional reprints may be purchased at cost price; the order is placed when the proofs are returned.

Please submit all manuscripts to

ACTA RADIOLOGICA

P O Box 7449, S-103 91 Stockholm, Sweden

ACTA RADIOLOGICA

FOUNDED IN 1971 BY GÖSTA FORSSFELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK FINLAND -

ONCOLOGY

RADIATION THERAPY PHYSICS AND BIOLOGY

EDITOR
ERIK LINDGREN

ADVISORY BOARD

Oncology Lars Gunnar Larsson
Radiation physics Gunnar Hejblum
Radiation biology Bernhard Tribukait

ASSOCIATE EDITOR
RUDOLF RUDHIE

EDITORIAL BOARD

Denmark G. Thomsen
Finland P. Virtanen
Norway T. Aakhus
Sweden L.-G. Larsson

- Granulosa and theca cell tumors—Incidence and occurrence of second primary tumors
- Prolactin secreting pituitary adenoma—Observations in irradiated patients
- Primary mucosal malignant melanoma—Appraisal of role of radiation therapy
- Hodgkin's disease—clinical stages I and II—Results of radical irradiation with or without chemotherapy
- Chloramphenicol toxicity in radiation disease
- Collimation of high energy electron beams
- Microdosimetry—II—Use of secondary electron emission to simulate two target models
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice—III—Variation of the chromosome counts of in vivo transplanted tumours in vitro cultures and retransplanted cultured cells
- Age and dose related carcinogenicity of ^{90}Sr
- Dose distribution around radium arrays used in the treatment of uterine carcinoma
- Irradiation injury of bone tissue—A vital microscopic method
- 161 BJORKHOLM F and SILVERSMAL C
- 169 DE SCHRYVER A VANDEKERCKHOF J and DEBRUYNE G
- 177 SON Y H
- 183 HIERNIB EGBAATH DURAND M et al
A MARFED HIERNISYNDG R
CHAUVERGNE J and LAGARDE C
- 193 POSPIŠIL M BENES L TRADLECEK L
VELCOVSKY V NETIKOV J and VIKREK
- 199 LAY I and BRAHME A
- 209 BURLIN T E and FORSBERG B J
- 215 BERGMAN H
- 223 NILSSON A BIERKE P WALINDER G and KARLSSON A
- 229 SHARMA P D and MACDONALD J C F
- 235 ALBREKTSSON T JACOBSSON M and THOMSEN

INSTRUCTIONS TO AUTHORS

ACTA RADIOLOGICA consists of two series: Diagnosis (red) including all medical imaging techniques and physiologic radiography and Oncology (blue) including radiation therapy, chemotherapy, nuclear therapy, radiation physics, and radiation biology. Only original manuscripts will be considered for publication, implying that the material has not already been published nor is intended for publication elsewhere. Extensive articles may be published as Supplements for which special conditions apply. Decisions on publication are based on the opinions of at least two reviewers (whose names are not revealed to the authors). The manuscripts are subject to editorial revision. The right is reserved to introduce such changes as may be necessary to make contributions conform to the editorial standards. The revised manuscript is submitted to the authors before typesetting. The journal does not hold itself responsible for opinions or statements expressed by the authors. The copyright resides with Acta Radiologica.

Manuscripts. Only manuscripts written in English are accepted. They should be typed on one side of the paper only, with double spacing (at least 1 cm between the lines) and margins of at least 4 cm on the left side and at the top. The manuscript should be submitted in duplicate and two complete sets of figures supplied. The name and address of the institution or hospital at which the work was carried out should be given on the title page. A summary normally not exceeding 100 words should be included. The author should also add an address to which correspondence can be directed and retain a copy of the manuscript for reference. A request for reprints should include full name, address and post code.

Abbreviations should be spelled out when first used in the text. Uncommon abbreviations and clinical jargon should be avoided. Footnotes are not accepted. Tables should be numbered in Arabic numerals and given on separate sheets, each with a short descriptive title. Legends for illustrations should be given on a separate sheet.

Contributions should be brief and concise, carefully revised before submission to exclude unessential matter. Alterations in the proofs are expensive, and with the exception of typographic errors will be charged to the author.

Units and Mathematics. The International System of Units (SI) WHO 1975 should be used. A zero should always precede a decimal fraction (i.e. 0.73). Mathematical expressions should be set out clearly. Make a circled notation in the margin to explain possibly ambiguous characters such as 1 (numeral 1 and letter l), 0 (zero and letter O or x (multiplication sign or letter x)). In handwritten equations, mark clearly e and c , u and v , and letters in which capitals and lower case are nearly indistinguishable when handwritten, also primes and apostrophes. Use the solidus (/) for fractions and $\exp()$ when the exponent is complicated. In displayed formulae, the horizontal fraction line is preferable. Composite numbers should be expressed as $n \times m$

and not as $m \times n$. Equations should be numbered sequentially in Arabic numerals in parentheses on the right side of the page.

Illustrations. Glossy prints, unretouched and unmounted, should be submitted. If the prints are not of a satisfactory standard, the author will be requested to submit the original film. Prints to be placed together should be of the same scale and of the same height to facilitate reproduction. The prints should have the same tonal relations as the original (for example, conventional angiographic films: white vessels on dark background; the reverse for subtraction films) with the patient's right to the reader's left. This is also valid for CT scans and other transverse images. Lettering and arrows are preferably marked lightly in pencil on the back, or removable from the prints.

Diagrams and graphs should be clearly drawn in black (Indian) ink on a white background and submitted as glossy prints or the original artwork. Lettering must be large enough to be legible after photographic reduction. Computer print-outs are usually not satisfactory.

Colour drawings or photographs are accepted if the costs are defrayed by the author(s).

Each illustration must be provided with a suitable short legend, typewritten on a separate sheet and comprehensible without reference to the text. It must include explanations of all abbreviations and arrows used.

The figure number and the name of the first author must be marked lightly in pencil and the top indicated on the back of each illustration.

References. The references should be arranged in alphabetical order with name(s) of author(s) followed by initials, full title of the article, and name of the journal as abbreviated in Index Medicus. The volume number, year of publication in parentheses, and number of the first page of the article should follow. All authors of the article must be given in the reference list. Reference to books and monographs should indicate the author, the title and edition of the book, the name of the publishers, and the place and year of publication.

Examples

BOUSEN E and ZSIGMOND M. Selective angiography of bronchial and intercostal arteries. *Acta radiol. Diagnosis* 3 (1965) 513.
KEITH A. Human embryology and morphology. 6th edition. P. 533. Arnold & Co. London 1943.

Reference in the running text to an article by one author (BOUSEN 1975), by two authors (BOUSEN & LIND 1975), by three or more authors. First author's name followed by et coll. (= co-workers) and not et al. (= and others)—(BOUSEN et coll. 1975). The accuracy of reference data is the responsibility of the author.

One hundred reprints of each article are supplied free of charge; additional reprints may be purchased at cost price provided the order is placed when the proofs are returned.

Please submit all manuscripts to

ACTA RADIOLOGICA

P O Box 7449, S-103 91 Stockholm, Sweden

ACTA RADIOLOGICA

FOUNDED IN 1921 BY GÖSTA FORSSELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK FINLAND NORWAY AND SWEDEN

ONCOLOGY

RADIATION THERAPY, PHYSICS AND BIOLOGY

EDITOR

ERIK LINDGREN

ADVISORY BOARD C

Oncology Lars Gunnar Larsson

Radiation physics Gunnar Hettinger

Radiation biology Bernhard Tribukait

ASSOCIATE EDITORS

ULF RUDHE ULF BERGVALL

EDITORIAL BOARD

Denmark G Thomsen S Kaae

Finland P Virtama L R Holsti

Norway T Aakhus E Poppe

Sweden L-G Larsson G F Saltzman

-
- | | | |
|---|-----|---|
| Theca-cell tumors—Clinical features and prognosis | 241 | BJÖRKHOLM E and SILFVERSWARD C |
| Sensitivity of cells in exponential and stationary growth phase to combined treatment with radiation and Quinacrine | 245 | MIDANDER J and LITTBRAND B |
| ✓ Calcitonin and mammary carcinoma | 251 | WAHLBY L and WESTMAN G |
| Effect of a screening programme on breast carcinoma incidence mortality and survival | 255 | SOINI I and HAKAMA M |
| Effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma in athymic nude mice | 261 | ROFSTAD E A LINDMO T and BRUSTAD T |
| Dose effect relationships in cervical and thoracic radiation myelopathies | 271 | HOLDORFF B |
| Chromosome counts of ^{90}Sr induced osteosarcomas in mice—IV—Variation of chromosome counts when using tumours of predetermined age for transplantation | 279 | BERGMAN H |
| Radiation sensitivity of DNA molecules in situ in normal and neoplastic tissues of mice | 285 | ONO T SAKAMOTO K and OKADA S |
| Induction of neoplasia by ^{140}Ba in mice | 293 | NILSSON A BIERKE P and BROOME KARLSSON A |
| Influence of thyroid hormones on erythropoiesis and radiation resistance in C57 black mice | 299 | MIKESKA J POSPIŠIL M NETIKOVA J and HOŠEK B |
| Electron and photon beams from a 50 MeV racetrack microtron | 305 | BRAHME A KRAEPELIEN T and SVENSSON H |

INSTRUCTIONS TO AUTHORS

ACTA RADIOLOGICA consists of two series: **Diagnosis** (red) including all medical imaging techniques and physiologic radio-graphy and **Oncology** (blue) including radiation therapy, chemo-therapy, nuclear therapy, radiation physics and radiation bio-logy. Only original manuscripts will be considered for publica-tion, implying that the material has not already been published nor is intended for publication elsewhere. Extensive articles may be published as Supplements for which special conditions apply. Decisions on publication are based on the opinions of at least two reviewers (whose names are not revealed to the authors). The manuscripts are subject to editorial revision. The right is reserved to introduce such changes as may be necessary to make contri-butions conform to the editorial standards. The revised manu-script is submitted to the authors before typesetting. The journal does not hold itself responsible for opinions or statements ex-pressed by the authors. The copyright resides with *Acta Radio-logica*.

Manuscripts. Only manuscripts written in English are accepted. They should be typed on one side of the paper only, with double spacing (at least 1 cm between the lines) and margins of at least 4 cm on the left side and at the top. The manuscript should be submitted in duplicate and two complete sets of figures supplied. The name and address of the institution or hospital at which the work was carried out should be given on the title page. A sum-mary normally not exceeding 100 words should be included. The author should also add an address to which correspondence can be directed and retain a copy of the manuscript for reference. A request for reprints should include full name, address and post code.

Abbreviations should be spelled out when first used in the text. Uncommon abbreviations and clinical jargon should be avoided. Footnotes are not accepted. Tables should be numbered in Arabic numerals and given on separate sheets, each with a short descriptive title. Legends for illustrations should be given on a separate sheet.

Contributions should be brief and concise, carefully revised before submission to exclude unessential matter. Alterations in the proofs are expensive and with the exception of typographic errors will be charged to the author.

Units and Mathematics. The International System of Units (SI) WHO 1975 should be used. A zero should always precede a decimal fraction (i.e. 0.123). Mathematical expressions should be set out clearly. Make a circled notation in the margin to explain possibly ambiguous characters such as *i* (numeral 1 and letter *l*), *0* (zero and letter *O*), *o* (multiplication sign or letter *x*). In handwritten equations, mark clearly *e* and *c*, *u*, *n* and *v* and letters in which capitals and lower case are nearly indistinguishable when handwritten, also primes and apostrophes. Use the solidus (/) for fractions and $\exp()$ when the exponent is complicated. In displayed formulae, the horizontal fraction line is preferable. Composite numbers should be expressed as $n \times m$

and not as $n \cdot m$. Equations should be numbered sequentially in Arabic numerals in parentheses on the right side of the page.

Illustrations. Glossy prints, unretouched and unmounted should be submitted. If the prints are not of a satisfactory standard and the author will be requested to submit the original film. Prints to be placed together should be of the same scale and of the same height to facilitate reproduction. The prints should have the same tonal relations as the original (for example, conventional angiographic films: white vessels on dark background; the re-verse for subtraction films) with the patient's right to the reader's left; this is also valid for CT scans and other transverse images. Lettering and arrows are preferably marked lightly in pencil on the back, or removable from the prints.

Diagrams and graphs should be clearly drawn in black (Indian) ink on a white background and submitted as glossy prints or the original art work. Lettering must be large enough to be legible after photographic reduction. Computer print-outs are usually not satisfactory.

Colour drawings or photographs are accepted if the costs are defrayed by the author(s).

Each illustration must be provided with a suitable short legend, typewritten on a separate sheet and comprehensible without re-ference to the text; it must include explanations of all abbrevi-ations and arrows used.

The figure number and the name of the first author must be marked lightly in pencil and the top indicated on the back of each illustration.

References. The references should be arranged in alphabetic order with name(s) of author(s) followed by initials, full title of the article and name of the journal as abbreviated in *Index Medicus*. The volume number, year of publication in parentheses and number of the first page of the article should follow. All authors of the article must be given in the reference list. Reference to book and monographs should indicate the author, the title and edition of the book, the name of the publishers and the place and year of publication.

Examples

BOISEN E. and ZIGMOND M.: Selective angiography of bronchia and intercostal arteries. *Acta radiol. Diagnosis* 3 (1965) 513.
KEITH A.: Human embryology and morphology, 6th edition. P. 533. Arnold & Co. London 1948.

Reference in the running text to an article by one author (BOISEN 1975), by two authors (BOISEN & LIND 1975), by three or more authors. First author's name followed by *et coll.* (= co-workers) and not *et al.* (= and others)—(BOISEN *et coll.* 1975). The accuracy of reference data is the responsibility of the author.

One hundred reprints of each article are supplied free of charge; additional reprints may be purchased at cost price provided the order is placed when the proofs are returned.

Please submit all manuscripts to

ACTA RADIOLOGICA

P O Box 7449, S-103 91 Stockholm, Sweden

ACTA RADIOLOGICA

FOUNDED IN 1971 BY GÖSTA FORSELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK FINLAND NORWAY AND SWEDEN

ONCOLOGY

RADIATION THERAPY PHYSICS AND BIOLOGY

EDITOR
ERIK LINDGREN

ADVISORY BOARD

Oncology Lars Gunnar Larsson
Radiation physics Hans Svensson
Radiation biology Bernhard Tribukait

ASSOCIATE EDITORS

ULF RUDHE ULF BERGVALL

EDITORIAL BOARD

Denmark G Thomsen S Kaae
Finland P Virtama L R Holsti
Norway T Aakhus E Poppe
Sweden L G Larsson G F Saltzman

-
- | | | |
|--|-----|---|
| Short term intra arterial Mitomycin C in hepatic metastases | 321 | MATTSSON W JONSSON K HELLEKANT C and HALLSTEN L |
| ^{32}P pyrophosphate in the treatment of persistent metastatic bone pain | 327 | WERNER B ISACSON C LUNDELL G LÖNNBECK C and SÖDERBORG B |
| Serum calcitonin in patients with osteolytic and osteosclerotic metastases from mammary carcinoma | 331 | NIELSEN H E and GADEBERG C CH |
| Palliative irradiation of brain metastases | 335 | TURALBA C I C EL MAHDIA M and PEEPLES W J |
| Transient intestinal ischaemia induced by degradable starch microspheres—Experiments in the cat | 343 | LOTE K FÖLLING M ROSENQEN B SVANES K and LEAVEN J |
| Radiation therapy of primary vaginal carcinoma | 353 | PIRTOLI L and SANTONI R |
| Relationship between histologic grading of head and neck tumours and regression after chemotherapy | 357 | JØRGENSEN K and SCHLICHTING J |
| Effects of acute gamma irradiation on spermatogenesis as revealed by flow cytometry | 361 | HACKER U SCHUMANN J and GÖHDE W |
| Uptake of serotonin liberated by irradiation of rabbits and mice | 369 | VENINGA T and LEMSTRA W |
| Induction of pituitary tumours by combination of oestrogenic hormones and ^{90}Sr | 373 | NILSSON A BIERKE P HARALDSSON I and BROOME KARLSSON A |
| Effects of proton irradiation of the lumbar intumescence on intra axonal transport of acetylcholine and cholinergic enzymes in rat sciatic nerve | 387 | BÖÖJ S DAHLSTRÖM A LARSSON P A ROSANDER K and ROSENGREN B |

(Continued overleaf)

Irradiation combined with Bleomycin treatment of synchronized cells in culture under oxic and hypoxic conditions	395	MIDANDER J, LITTBAND B and EDSMYR F
Nuclear imaging of pulmonary metastases in thyroid carcinoma	401	WORM A M, HOLTEN I and TAANING E
Microprocessor system for tracking isodensity lines in film dosimetry	405	VAN DER LAARSE R and DE GANS J
Photon energy spectra of a heavily filtered 300 kV therapy unit	411	CHEN T S, KASE K R and BJARNGARD B

SUBSCRIPTIONS

Acta Radiologica		in Scandinavia	outside Scandinavia
Diagnosis — Medical Imaging and Physiologic Radiology (red)	} both vols	Sw Kr 400 —	Sw Kr 475 —
Oncology incl Radiation Therapy Physics and Biology (blue)			
Diagnosis	one vol	Sw Kr 760 —	Sw Kr 275 —
Oncology	one vol	Sw Kr 190 —	Sw Kr 205 —

All rates include regular mailing costs (surface mail)

*All communications regarding advertising, subscription
change of address, etc should be sent to
Acta Radiologica, P O Box 7449, S-103 91 Stockholm, Sweden*

ACTA RADIOLOGICA

FOUNDED IN 1971 BY GÖSTA FORSSELL

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK FINLAND NORWAY AND SWEDEN

ONCOLOGY

RADIATION THERAPY¹²³¹ PHYSICS AND BIOLOGY

EDITOR

ERIK LINDGREN

ADVISORY BOARD

Oncology Lars Gunnar Larsson

Radiation physics Hans Svensson

Radiation biology Bernhard Tribukait

ASSOCIATE EDITORS

ULF RUDHE ULF BERGVALL

EDITORIAL BOARD

Denmark G Thomsen S Kase

Finland P Virtama L R Holsti

Norway T Aakhus E Poppe

Sweden L-G Larsson G F Saltzman

✓ Superfractionated irradiation combined with low doses of Bleomycin in the treatment of oral carcinoma
Radiation therapy of glottic carcinoma stage I

Advanced carcinoma of the tonsil—Treatment results

✓ Radiation therapy of nasopharyngeal carcinoma
Fast neutron teletherapy for advanced carcinoma of hypopharynx and supraglottic larynx

Hypothyroidism following ¹³¹I therapy for hyperthyroidism in relation to immunologic parameters

Thyroid treatment and its possible influence on occurrence of malignant tumors after diagnostic ¹³¹I
Bone abnormalities in patients with medullary carcinoma of the thyroid

Invasive squamous cell carcinoma of the uterine cervix—I—Definition of parameters in a histopathologic malignancy grading system

Survival in carcinoma of the uterine cervix correlated to primary treatment results

417 LINDHOLM C LITTEBRAND B and LÖFROTH P O

421 JOSE B CALHOUN D L MOHAMMED A and TOBIN D A

425 PETROVICH Z KUISK II JOSE L BARTON R and RICE II

433 CHANG C LIU T CHANG Y and CAO S

439 LARAMORE G E JOHNSON J GRIFFIN T W TONG D GROUDIN E M T KURTZ J M RUSSELL A H and PARKER R G

449 LUNDELL G and HOLM L E

455 HOLM L E

461 RASMUSSEN B

467 STENDAHL U WILLÉN H and WILLÉN R

481 JINENEZ J ALERT J BELDARRAIN L MONTALVO J and ROCA C

(Continued overleaf)

Irradiation combined with Bleomycin treatment of synchronized cells in culture underoxic and hypoxic conditions	395	MIDANDER J LITTEBRAND H and EDSMYR F
Nuclear imaging of pulmonary metastases in thyroid carcinoma	401	WORM A M HOLTEN I and TAANING E
Microprocessor system for tracking isodensity lines in film dosimetry	405	VAN DER LAARSE N and DE GANS J
Photon energy spectra of a heavily filtered 300 kV therapy unit	411	CHEN T E KASE K R and BJÄRNGÅRD B E

SUBSCRIPTIONS

Acta Radiologica		in Scandinavia	outside Scandinavia
Diagnosis — Medical Imaging and Physiologic Radiology (red)	both vols	Sw kr 400 —	Sw kr 425 —
Oncology incl Radiation Therapy Physics and Biology (blue)			
Diagnosis	one vol	Sw kr 260 —	Sw kr 275 —
Oncology	one vol	Sw Kr 190 —	Sw Kr 205 —
All rates include regular mailing costs (surface mail)			

*All communications regarding advertising, subscription,
change of address etc should be sent to
Acta Radiologica, P O Box 7449, S-103 91 Stockholm, Sweden*

EDITORIAL

With this issue Acta Radiologica begins publication in a new form after having had the same appearance and been produced by the same processes ever since it was founded in 1921 by Gosta Forssell. The design of the cover for the individual numbers has however been changed the table of contents being moved in 1951 to the front page in order to make it easier for readers to obtain information on the articles in the issue without having to open it. And in 1963 the journal was divided into two series one on diagnosis and one on radiation therapy, physics and biology.

It is of great importance that the illustrations in a periodical presenting radiologic material should be of the highest quality. If a reader is able by examining the illustrations to satisfy himself that the author has interpreted the images correctly he will also find it easier to evaluate the conclusions drawn in other words form a better opinion regarding the value of the article. Even with blocks of high quality it has sometimes been necessary because of the size of the journal to present illustrations which in some cases have been too small with the result that some of the details could not be readily distinguished. With increasing refinement of the imaging techniques and as ever smaller details have proved to have diagnostic significance a sense of dissatisfaction with the format of the journal has gradually developed. A change over to a larger size has in fact been under consideration for several years but has been deferred until a change to a more modern method of production could be effectuated at the same time.

As from the present issue the journal will be produced by means of modern computer generated phototypesetting and offset printing. Other periodicals have earlier adopted the offset technique but in the opinion of the editors of this journal images produced with this process have only recently attained a quality that can be considered acceptable

for use in radiologic publications. Thus the change to offset was not made until comparative trials had brought conviction that the new method for image reproduction has reached a stage giving results comparable to those obtained with the old block making system.

Apart from the change in appearance and the new method of production the principles of the journal will remain unchanged. This means that the two series will continue to be published also in the future. The diagnosis series will as before be a journal of general radiology with sidelines on some special fields such as neuroradiology and pediatric radiology. In the future as in the past considerable attention will be paid to the layout in order to ensure that the articles are presented in a clear and esthetically attractive form.

One of the reasons why Acta Radiologica was instituted was the desire to have a forum where experiences and results of research in radiology and allied fields in the Nordic countries could be published so that readily available information could be obtained regarding developments, trends and future prospects in these countries. As the speech communities covered by these countries are small the articles had to be published in one of the major languages of the world if they were to be available to readers in other areas. In the beginning papers in English, German and French were accepted. However English has gradually become the dominant international language in the medical world holding a position almost comparable to that of Latin in the world of science during the Middle Ages and the centuries immediately following that period and the journal therefore subsequently adopted the policy of publishing articles only in English. Summaries in German and French were retained but from this issue onwards they will be abandoned since it may be considered hardly possible these days to follow developments in any branch of medicine without a

knowledge of English *Acta Radiologica* will therefore from now on be published exclusively in English.

Although the journal was originally intended to be a forum mainly for the Nordic nations its pages have always been open to authors from other countries as well. Many important articles by non Nordic writers have also been gratefully accepted for publication. It is hoped that these contributions will not only continue but also increase in number. Problems will thereby receive more all round treatment and comparisons with other points of view facilitated which should be to the mutual advantage of both *Acta Radiologica* and its readers.

In recent years new diagnostic methods based on imaging techniques have been introduced ultrasound, scintigraphy, computer tomography and possibly still others such as magnetic resonance will appear in the future. Opinions differ it is true regarding the appropriate speciality to which these methods should belong. *Acta Radiologica* holds most emphatically that all imaging techniques should be concentrated to the radiologic departments where the basic skills are centered. Furthermore the methods are as a rule such that the solution of a diagnostic problem is seldom gained by

one method alone. The methods are supplementary to each other as well as to conventional radiology. Thus the most suitable way to achieve this is to assemble them all in one institution. In accordance with this opinion *Acta Radiologica* has published and will continue to publish articles on all imaging procedures. In order to emphasize more clearly than previously the fact that the diagnosis section of the series is a journal of medical imaging information to this effect has been added to the first page of the cover. It further appears that the journal will also accept reports on physiologic sequences analysable by radiologic methods in the widest sense.

Like other clinical specialities oncology needs the methods offered by diagnostic radiology but otherwise they have little in common. They should accordingly be practised by different specialists. As a consequence of this *Acta Radiologica* as the only radiologic periodical has run and will continue to run two series: one diagnosis and one oncology the latter comprising not only radiation therapy but also chemotherapy and radiation physics and biology.

With this declaration of its programme *Acta Radiologica* is presented to the readers in its new form.

LARYNGEAL CARCINOMA

IV Analysis of treatment results using the Cohen model

M. HJELM HANSEN

This report presents an analysis of treatment results in which the biologic effect of irradiation is estimated by using the Cell Population Kinetic Model proposed by COHEN (1971). The reader is encouraged to review all cited papers by COHEN as well as the one by SACHER & TRUCCO (1966) in order to gain an understanding of the details of this model.

Material, Methods and Definitions

The material consisted of 308 patients (94 supraglottic, 211 glottic, 3 subglottic) treated by primary irradiation for laryngeal carcinoma in the period August 1963 to August 1972. Clinical details regarding the total material of 359 patients from which the analysis group has been selected are given in Part I (JØRGENSEN et coll. 1979). Details regarding the analysis group of 308 patients as well as an analysis of treatment results using the Ellis model are given in Part II (HJELM HANSEN et coll. 1979) and the treatment results in relation to a microscopic score are found in Part III (LUND et coll. 1979). The clinical data on which the analysis is based refer to December 1976.

Statistical method. The chi square test was used to test for correlations when frequencies are given as minimum or estimated values. If frequencies are calculated by use of the life table method (Fig. 4) the logrank test was used (PETO et coll. 1977).

Minimum absorbed dose (D_{min}) to the tumour was taken to be 95 per cent of the calculated central absorbed dose (D_c). For larger tumours this was a

claim for inclusion in the analysed group and for smaller tumours (well within the fields) the build up and build down of dose at the intersection of air and tissue (trachea and laryngeal cavity) will cause the minimum absorbed dose to be approximately 5 per cent lower than the central absorbed dose obtained in ordinary dose planning (KOSKINEN & SPRING 1973). Minimum absorbed dose is used in analysis of local recurrence.

Maximum absorbed dose (D_{max}) is defined as the absorbed dose at the central axis in a depth of 0.5 cm. Maximum absorbed dose was used in the analysis of complications. Usually with the technique used today very little difference occurs between D_c and D_{max} . However in the early series some patients received treatment with alternating fields and while D_c is associated with a number of equal fractions D_{max} is associated with alternating large and small fractions which may produce a different biologic effect than a number of equal fractions.

The Cohen model is based on calculation (separately for each individual tissue in question) of survival fraction obtained after each individual fraction of irradiation and of repopulation during the time intervals between the individual fractions. The survival curve is assumed to be given by the equation

$$S = e^{-D^j} [1 - (1 - e^{-kD})^j] \quad (1)$$

where S is the surviving fraction, D is the absorbed dose (tumours D_{min} , normal tissues D_{max}) and j is

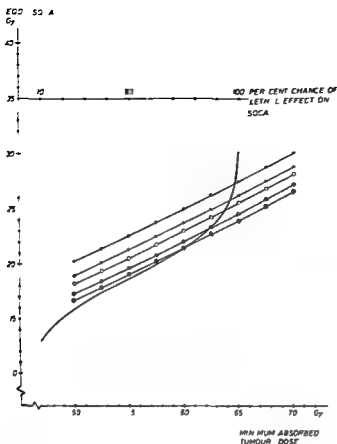


Fig 1 Graph for determination of EQD1 SQCA and prognosis according to the Cohen model used on a schedule of 30 fractions delivered in 41 days. Tumour size: \circ 1 cm, \bullet 2 cm, \square 3 cm, \triangle 5 cm, \bullet 7 cm.

and N are constants which must be specified for the tissue in question.

After termination ($T=0$) of a treatment resulting in a surviving fraction of $S(0)$ repopulation is thought to take place after the logistic formula of SACHER & TRILCCO

$$S(T) = \frac{H S(0)}{S(0) + (H - S(0))e^{-LHT}} \quad (2)$$

$S(T)$ is the surviving fraction at time T . H is the possible maximum value that S can reach (depends on previous treatment) and L is a growth constant to be specified for each individual tissue. (In some recent publications on the model COHEN is omitting the factor H in the exponential expression which however may change the value of L .)

Although eq 2 is derived for normal tissues only (in which case H has a maximum value of 1) it is used for tumour tissues as well by allowing H to exceed the value of 1 i.e. tumour growth beyond the initial size is possible.

Procedure of calculation

(1) Previous treatment has resulted in a surviving fraction of size $S(0)$. (This means $S(0)=1$ if no previous treatment has been given.)

(2) During the time from termination of previous treatment to next fraction repopulation will take place and cause the surviving fraction to increase according to eq 2. In order to use this equation must be known.

For tumour tissues H is a constant in the range 2 to 20 while for normal tissues H depends on previous treatment and is calculated from the equation

$$H = e^{G \frac{T}{\pi}} S_1 \quad H \leq 1$$

where n fractions with the individual effects $S_1, S_2, S_3, \dots, S_n$ (eq 1 with $D=D_1, i=1, 2, 3, \dots, n$) constitute the previous treatment and G is a constant to be specified for the tissue in question. If the value of H calculated from eq 3 is greater than 1 then $H=1$.

(3) The surviving fraction immediately before next fraction is then obtained (S_1).

(4) The separate effect of next fraction (S_2) calculated from eq 1.

(5) The total surviving fraction immediately after this last fraction is then $S(0)=S_1 \times S_2$.

(6) Sequence 1 to 5 is repeated as long as further fractions are given.

(7) After termination of the treatment a final value (S_T) of surviving fraction is obtained also given by

$$S_T = \frac{1}{\pi} \frac{H(i) S_{i+1}}{S(i) + [H(i) - S(i)]e^{-L(i+1)V(i)}}$$

where $H(i)$ is the value of H after fraction No. i (eq 3). $S(i)$ is the total surviving fraction immediately after fraction No. i while $V(i)$ is the interval between fraction No. i and $i+1$ ($H(0)=S(0)=1$). S_{i+1} is the separate effect of fraction No. $i+1$ (eq 1).

Correction for tumour size is performed by multiplying S_T by the factor Z^Y where Z is the tumour size in cm and Y is a constant to be specified for each individual type of tumour. This correction is merely a mathematical convenience utilizing the fact that if a certain survival fraction is obtained a larger number of tumour cells will be left in a large tumour than in a smaller one. The prognosis of the larger tumour is assumed to be similar to the prognosis of the smaller tumour treated less intensively.

Table 1

Values of the constants used in the present analysis

	G	j rad ⁻¹	k rad ⁻¹	L day ⁻¹	N	Y	μ_a	σ_a
SKIN	8.72	0.00415	0.00486	0.34	13.0	0.57	4.70	1.93
VASC	18.00	0.00190	0.00370	0.23	6.6	0.33	4.00	2.00
GUT	18.00	0.00160	0.00470	0.13	21.1	—	4.70	2.20
SQCA	1.50	0.00480	0.00620	0.07	11.0	2.00	7.87	2.01

The reason for including the parameter GUT (significant long term sequelae in the gastrointestinal tract) although this has no relevance for laryngeal carcinoma, is that this parameter appears in the discussion of the split-course technique.

Table 2

Values of the parameters EQD1 SQCA and EQD1 VASC in a treatment schedule of 30 fractions in 41 days compared with the values of the parameter $PT_{50\%}$ (Ellis model)

Central absorbed dose (Gy) D	Minimum absorbed dose (Gy) $D_{95\%}$	EQD1 SQCA (based on D_{min})		EQD1 VASC (based on D)		$PT_{50\%}$ (based on D) ret
		Tumour size (cm)	Gy	Field size (cm)	Gy	
57.00	54.15	1	22.9	6	12.3	1.612
		3	0.1	8	14.4	
		5	19.3	10	16.2	
60.00	57.00	1	23.6	6	13.4	1.746
		3	21.5	8	15.6	
		5	20.6	10	17.7	
63.00	59.85	1	25.0	6	14.4	1.893
		3	23.0	8	16.9	
		5	22.1	10	19.2	
66.00	62.70	1	6.4	6	15.6	2.025
		3	24.4	8	18.2	
		5	23.5	10	21.2	

(i.e. to a higher survival fraction) in which case a similar number of tumour cells would be left in the two tumours.

In the present analysis of local recurrence (Figs 2-3) the tumour size is taken to be the largest dimension of the tumour (an integer number of cm) which means that a tumour of dimensions 3 cm × 1 cm is corrected as a tumour of size 3 cm × 3 cm. Since such two tumours obviously contain different numbers of cells, some problems may arise.

The difference in the value of EQD1 SQCA for a 5 cm × 5 cm tumour and a 5 cm × 3 cm tumour is about 0.5 Gy. However, if the width of the tumour is much smaller than the length (as typical in glottic

stage I) the difference in the value of EQD1 SQCA may be 1.0 to 2.0 Gy. At the steepest part of the theoretical response curve (Fig. 1) this may be associated with a difference in local recurrence of 10 to 20 per cent, which indicates that correct assessment of tumour size is an important point, especially with respect to smaller tumours.

The analysis presented in Fig. 4 contains the assumption that tumours in glottic stage I of size (length) 1 cm, 2 cm and 3 cm in fact represent tumour areas of 1 × 0.3 cm², 2 × 0.4 cm² and 3 × 0.5 cm². The size of these tumours is then taken to be the diameter of circles with areas 0.3 cm², 0.8 cm² and 1.5 cm² respectively.

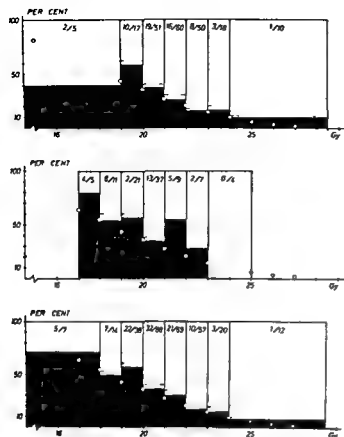


Fig 2 Observed frequencies of local recurrence as function of EQD1 SQCA (Gy) compared with the Cohen model (white circles). Black columns indicate minimum frequencies while stippled lines indicate estimated frequencies. Upper: Glottic carcinoma (211 patients). Middle: Supraglottic carcinoma (94 patients). Lower: Glottic and supraglottic carcinoma (305 patients). Correlations (estimated frequencies) in parentheses. Glottic— $p < 0.003$ $f=4$ ($p < 0.001$ $f=4$). Supraglottic— $p < 0.01$ $f=1$ ($p < 0.025$ $f=2$). Total— $p < 0.0005$ $f=4$ ($p < 0.0005$ $f=4$).

Correction for field size. During the computation of S_F a corrected value (U_i) of the absorbed dose (D_i) is used. The corrected value is calculated from

$$U_i = \left(\frac{d}{10} \right)^Y D_i \quad (5)$$

(suffix i is used because the individual fractions may be of different size) d is the diameter of the field in cm (10 is the side of the equivalent square field) and Y is a constant to be specified for the normal tissue in question. This correction is a mathematical convenience similar to the correction for tumour size.

(8) Having performed the corrections the equivalent single dose (EQD1) may be calculated as the value of D which inserted into eq 1 yields the final corrected surviving fraction ($S_{F \text{ corr}}$)

$$S_{F \text{ corr}} = e^{-k \text{EQD1}} (1 - (1 - e^{-k \text{EQD1}})^N)$$

and since $k \text{EQD1} \ll 10$ $e^{-k \text{EQD1}} \ll 1$ and the binomial

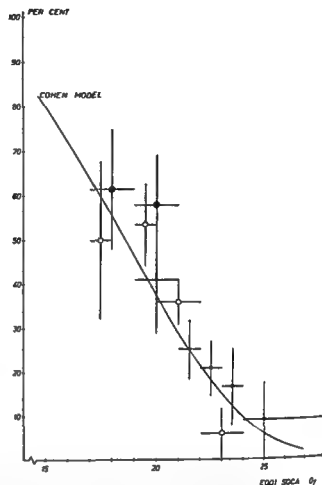


Fig 3 Observed frequencies (minimum values) of local recurrence according to tumour size. The standard deviations are calculated from the binomial distribution. Correlations 3 to 4 cm $p < 0.025$ $f=2$. Size of tumour: \bullet 1 to 2 cm, \circ 3 to 4 cm, \bullet 5 to 7 cm.

expansion $(1 - e^{-k \text{EQD1}})^N \sim 1 - N e^{-k \text{EQD1}}$ may be used. Thus $S_{F \text{ corr}} \sim N e^{-k \text{EQD1}}$ or

$$\text{EQD1} = \frac{2.303(\log_{10} N - \log_{10} S_{F \text{ corr}})}{J+k} \quad (6)$$

(9) The answer from the Cohen model may then be to state the surviving fraction the equivalent single dose or the probability of a specific biologic effect which is expressed as a cumulative normal distribution (function of $Q = -\log_{10} S_{F \text{ corr}}$) by specifying the mean value (μ_Q) and the standard deviation (σ_Q).

COHEN has estimated the values of the constants J , k , N , G , L , Y , μ_Q and σ_Q for a number of tissues among which are normal skin (SKIN), vascular stroma (VASC) and squamous cell carcinoma (SQCA).

The biologic effects in question are SKIN, normal

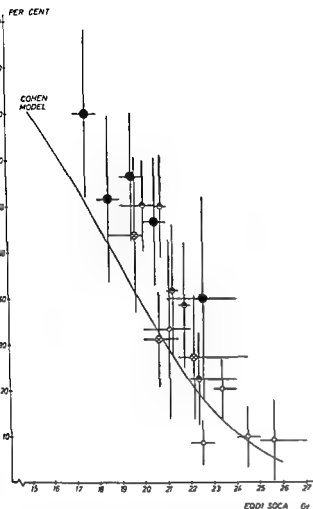


Fig 4 Observed frequencies (10 year life table values) of local recurrence according to tumour location and stage (UICC 1972). The standard deviations are calculated from the Greenwood approximation. Larynx (304 patients) ○ glottic stage I ⊕ glottic stage II and III ⊗ supraglottic stage I and II ● supraglottic stage III and IV. Correlation glottic stage II and III $p < 0.05$ $t=2$.

skin tolerance corresponding to a dry desquamative reaction VASC late effect in the vascular stroma such as telangiectasis or endarteritis SQCA long term local control of epidermoid carcinoma. The values of the exponents used in the present analysis appear in Table I.

It should be stressed that such values are subject to change since they are derived from statistical analysis of clinical data and may be recalculated when more reliable data are provided. All calculations in the present analysis have been performed on a computer using a version of COHEN's RADLOG program (1973) kindly provided by COHEN.

Example Some results of calculation using the Cohen model (SQCA) on a fractionation schedule of

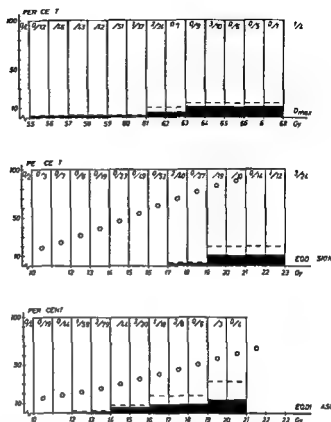


Fig 5 Observed frequencies of late edema as a function of D_{max} EQD1 SKIN (D_{max}) and EQD1 VASC (D_{max}). Black columns indicate minimum frequencies while stippled lines indicate estimated frequencies. The figure includes 3 patients in the subglottic group. Circles indicate the risk of dry desquamative reaction (SKIN) and of telangiectasis (VASC) according to the Cohen model.

30 fractions delivered in 41 days appear in Fig 1.

A minimum absorbed dose of 55 Gy delivered to a tumour of size 1 cm results in a value of EQD1 SQCA ~ 22.5 Gy which according to the model is associated with a chance of lethal effect of approximately 82 per cent. The same treatment given to a tumour of size 2 cm would result in EQD1 SQCA ~ 21.2 Gy corresponding to approximately a 72 per cent chance of local control while the same treatment given to a tumour of size 7 cm would result in EQD1 SQCA ~ 19 Gy and 53 per cent chance of local control.

Table II shows values of EQD1 SQCA and EQD1 VASC for some combinations of tumour size and field size in the treatment schedule of 30 frac

Table 3

Comparison between observed and calculated frequencies

Period (most common schedule)	Observed frequency (minimum)	Calculated frequency (per cent) (the working hypothesis)
August 1963–June 1970 (57 Gy/30 frac/41 days)	Local recurrence Late edema	76/208–36.5±3.3% 57/108–2.4±1.1%
July 1970–Aug. 1972 (60 Gy/30 frac/41 days)	Local recurrence Late edema	25/97–25.8±4.4% 6/97–6.2±2.4%
		33 6 23 10

tions delivered in 41 days. The values are also compared with the values of the parameter PT_{1000} used in the Ellis model.

Results

Local recurrence A total of 103 cases (42 supraglottic, 59 glottic and 2 subglottic) of local recurrence were analysed (as of December 1976). The observed frequencies of local recurrence in the glottic, the supraglottic and the total group as function of EQD1 SQCA (D_{min}) are given in Fig. 2. Black columns indicate minimum frequencies, while stippled lines indicate estimated values (glottic stage I corrected for death from intercurrent disease within 6 years, supraglottic stage III and IV corrected for death within 2 years and all other groups corrected for death within 4 years).

A very strong correlation exists between local recurrence and EQD1 SQCA. This correlation is in fact very close to the one proposed by COHEN, although the observed frequencies of local recurrence are usually slightly higher than predicted from the model. However, this could easily be corrected merely by adjusting the values of μ_0 and σ_0 .

Automatically, a bias is presented in Fig. 2, since larger tumours (i.e. advanced stages) will appear with lower values of EQD1 SQCA, while smaller tumours will appear with larger values of EQD1 SQCA, if the actual treatments are equal. This is one of the advantages of the Cohen model, since it allows simultaneous analysis of the whole group irrespective of tumour location and stage. Theoretically, any tumour treated to a specific level of EQD1 SQCA should have a prognosis corresponding to this level (Fig. 1).

Observed frequencies (minimum values) of local recurrence according to tumour size (largest dimension of the tumour) appear in Fig. 3, which shows that observed frequencies—at least for tumours of size less than 5 cm—follow the model. However, since minimum frequencies are approximately 90 per cent of the estimated frequencies, it is evident that observed values are slightly higher than predicted from the model.

Observed frequencies (10 year life table values) according to tumour location and stage are given in Fig. 4. As discussed under correction for tumour size, an assumption about the size of glottic tumours stage I is made in this analysis.

When the standard deviations are considered, it must be admitted that it is possible that equal values of EQD1 SQCA does in fact mean equal risk of local recurrence irrespective of tumour location and stage. It is also evident that the true response curve is most likely steeper than indicated by the Cohen model. The correction as to size in glottic tumours stage I changes the distribution of the total in a way that disturbs the frequency figures in Fig. 2. However, the correlation is still valid.

Significant correlations between local recurrence and EQD1 SQCA are found for tumours of size 3–4 cm ($p < 0.025$, $f = 2$) and for the group of glottic tumours stage II and III ($p < 0.05$, $f = 2$) as was the case in the analysis using the Ellis model (Part HJELM HANSEN et al.).

Late edema A total of 11 late edemas were observed (9 in the supraglottic group and 2 in the glottic group). Fig. 5 gives the observed frequencies analysed against D_{min} , EQD1 SKIN (D_{max}) and EQD1 VASC (D_{max}). Estimated values are based on a correction for local recurrence and death within one year.

Significant differences occur in the analysis in which the parameter is corrected for field size.

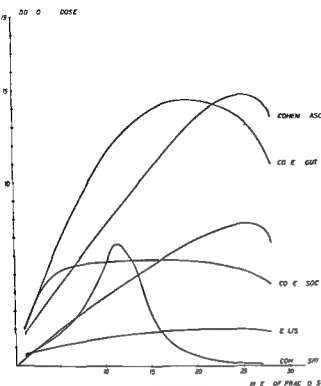


Fig 6 Calculations concerning split-course technique using different fractionation models. First values are calculated for the schedule 60 Gy/30 fractions/41 days. Then an interval of 30 days is inserted in the treatment period after N fractions. The additional dose (Gy) means the dose that must be added to the last 30 N fractions delivered after the interval in order to obtain the values first calculated, i.e. in order to obtain equal biologic effect in the split-course schedule and the continuous schedule (according to the model in question). The field size when needed is considered to be 10 cm. Number of fractions (X axis) is the number of fractions delivered before the treatment interval.

Thus (based on estimated frequencies) at EQD1 VASC=13 Gy (1/106=0.9 per cent 10/130=7.7 per cent $p<0.025$) and at EQD1 SKIN=17 Gy (0/134=0 per cent 11/102=10.8 per cent $p<0.001$).

The Cohen model for SKIN estimates the frequency of dry desquamative reaction as a function of EQD1 SKIN. This estimate seems to be far from the observed frequencies of late edema. The model for VASC predicts the risk of late effects in the vascular stroma (telangiectasis) and the risk seems to be approximately 20 per cent higher than the observed frequencies of late edema.

Pharyngo-cutaneous fistula. A total of 16 pharyngo-cutaneous fistulas have been observed all after total laryngectomy for local recurrence. These are not clearly correlated to treatment level. If the frequency of fistulas is calculated among the 72 patients on whom a total laryngectomy was performed a decreasing or constant frequency is found as func-

tion of D_c , D_{max} and EQD1 SKIN (D_{ma}) while an increasing frequency occurs as function of EQD1 VASC (D_c), EQD1 VASC (D_{max}) and EQD1 SKIN (D_c).

Although it must be admitted that fistulas may develop for other reasons than previous irradiation an increasing risk with treatment level and field size should be expected. The present material slightly indicates that the development of fistulas depends more on field size than on absorbed dose level. The observed complications seems to be most satisfactorily related to the parameter EQD1 VASC.

If fistulas, necrosis and late edema are analysed together (which however should not be done) against the parameter EQD1 VASC (D_{max}) the frequencies are EQD1 VASC less than 12 Gy (2/67=3.0 per cent) 12-14 Gy (11/138=8.0 per cent) 14-16 Gy (8/64=12.5 per cent) greater than 16 Gy (7/39=17.9 per cent). These frequencies are very similar to the estimated frequencies of late edema (Fig 5).

Working hypothesis. The Cohen model for SQCA will correctly estimate the frequency of local recurrence. The Cohen model for VASC will overestimate the frequency of late edema by 20 per cent.

Optimum treatment level. If the schedule is still restricted to 30 fractions 41 days an optimum value of absorbed dose may be estimated to be $D \sim 65$ Gy ($D_{min} \sim 62$ Gy). This optimum level appears to be only slightly dependent on the actual combination of tumour size and field size. By optimum is meant that the highest possible number of patients will pass the treatment without local recurrence or late edema. Other criteria—such as to aim at the lowest number of total laryngectomies—might as well be justified.

The effect of changing the absorbed dose from $D = 60$ Gy to $D = 65$ Gy is calculated to be a reduction in the frequency of local recurrence from 23 per cent to 13 per cent with an increase in the frequency of late edema from 10 per cent to 19 per cent. (This is based on the distribution of tumour size and field size from the present material (Table 3). Although this result would represent an improvement there is evidence suggesting that the reduction in local recurrence may be obtained with no increase in late edema. This result could possibly be achieved by the use of split-course technique.

Conversion to split course schedule. Several authors (HOLSTI 1967 1969a b 1971 SAMBROOK 1962 1963a b SCANLON 1960 1968) claim that an interval (usually of 2 to 4 weeks) inserted after half

of the treatment period) will not affect the probability of local control if the absorbed dose is increased by approximately 10 per cent to compensate for tumour growth during the treatment interval and even then the patient will tolerate the treatment better. The opinion of these authors is based on the treatment of several thousands of patients including some with laryngeal carcinoma.

Fig. 6 illustrates how the Cohen model for GUT, VASC, SKIN and SQCA, the Kirk model (KIRK et coll. 1975) and the Ellis model (ELLIS 1968, 1969, WINSTON et coll. 1969) would predict the effect of an interval of 30 days introduced in the treatment schedule (60 Gy/30 fractions/41 days).

The Cohen model (SQCA) predicts that approximately a 10 per cent higher absorbed dose should be given in order to obtain equal probability of local control on squamous cell carcinoma. This appears to be fairly independent of the position of the interval (and of tumour size).

As for the effect in normal tissue, the Ellis model is not in accordance with the experience concerning the split-course technique; the Kirk model is so to some degree, whereas the Cohen model is so to a high degree with respect to the tissues in the gastrointestinal tract (GUT) and the vascular stroma (VASC). (Rectal complications have been analysed in 664 patients with carcinoma of the uterine cervix (HJELM HANSEN & SELL 1977). Excellent agreement with the Cohen model was found and the split-course technique based on the Cohen model has been adopted since January 1978 with good results.) The Cohen model used for SKIN behaves differently and in fact (like the Ellis model) indicates that the frequency of complications increases in the split-course schedule. Except when the parameter EQD1 SKIN is used, the interval should preferably be placed in the last half of the treatment period.

The opinions based on the split-course schedule mostly concern the acute reactions (patients tolerate the treatment better) while the Ellis model is based on late reactions. However, the Cohen model (VASC) is also based on late reactions (late effects in the vascular stroma such as telangiectasis or end arteritis) and the disagreement between the two models is an obvious matter for analysis.

If the calculations are based on the working hypothesis and if the size of one fraction is restricted to the usual value ($D_e = 2.00$ Gy) and the treatment interval to 21 days, the optimum treat-

ment ($D_e = 65$ Gy/30 fractions/41 days) converted to split course technique becomes $D_e = 72$ Gy/36 fractions/70 days with an interval of 21 days inserted after 20 fractions. This schedule has been used during 1978 when the size of the equivalent square field did not exceed 8 cm. When larger fields were used the schedule was reduced by 3 fractions.

In the high level schedule the value of EQD1 SQCA will be greater than 22 Gy and the value of EQD1 VASC will be less than 12 Gy. Thus according to the working hypothesis, the frequency of local recurrence (Fig. 2) should be less than 70 per cent (the overall frequency is calculated to 13%) while the frequency of late edema (Fig. 5) should be very low since the previous experience (although without split-course) is 0/67 (confidence limits 0.00–7.60%).

However, based on other parameters than EQD1 VASC, different predictions are found indicating that the frequency of late edema may increase to as much as 30 per cent.

Preliminary experience with the split course schedule. From the experience gained during the first year (67 patients) the impression is that the patients tolerate the split-course schedule in approximately the same way as the old schedule, i.e. acute effects are similar in spite of a considerable increase of absorbed dose. The patients like the rest period and most patients report that any reaction felt at the beginning of the interval disappears during its course. However, slight reactions often reappear within the first week of continued treatment.

It is too early for any statement about tumour response and late reactions. However, two surprisingly strong reactions have been observed (one required a total laryngectomy) and in order to reduce the risk it has been decided to use a reduced schedule from January 1979 (omitting the last two fractions) and await the analysis of local recurrence among the 67 patients with the high level split course schedule during 1978.

Discussion

COHEN (1978b) discusses some of the limitations and possibilities of the model and the reader is referred to this discussion.

The model (SQCA) has been tested on the present material and on 664 patients with carcinoma of the uterine cervix and although the analysis of this last material is much more complex, the same tendency

of excellent agreement with the model was found. In both materials the model is predicting slightly lower frequencies of local recurrence than observed. Since the present data for high values of EQD1 SQCA (>22 Gy) would justify only a slight adjustment of the model and since this part of the response curve is the important part with respect to optimization, no adjustment will be proposed at this stage. The model has only been tested on schedules with 5 fractions per week with only a few (and small) unplanned intervals.

The optimum schedule calculated in the present analysis could (according to the model) be better if the restriction of 5 fractions per week could be disregarded. Comparison with results from the literature is difficult because information about tumour size is lacking. However, at least a qualitative comparison could be made with two randomized trials comparing 5 fractions per week with 3 fractions and 1 fraction per week, respectively.

One of these is the trial run by the British Institute of Radiology since 1963 of 5 versus 3 fractions per week in a number of different schedules (Second Progress Report 1968). In the Sixth Interim Progress Report (1978) on this test it is reported that among 687 patients treated, those who received 5 fractions per week have a slightly higher ($\sim 5\%$) local recurrence free rate.

According to the Cohen model, some of the schedules used (long overall time) would be better (5–10%) with 5 fractions per week, while others would be equal or slightly better (0–3%) with 3 fractions per week. Thus it is in excellent agreement with the model that only a small difference has been observed. The largest difference has been observed among the smallest tumours (cords mobile), but it should be noted that any odd distribution of tumour size in the two groups would give rise to a much larger difference than the one caused by the fractionation. According to the model, the schedules with 5 fractions per week should give rise to more (5 to 10%) complications, which, however, have not been observed.

The second trial of 5 versus 1 fraction per week was presented by DVIVEDI *et al.* (1978) and includes 341 patients, among which 202 patients were head and neck cases. Although the follow-up is incomplete and short, the authors conclude that the tumour response is equal and that 1 fraction per week is tolerated better. The schedules in question were 30×2.00 Gy/41 days and 6×6.70 Gy/36 days.

The Cohen model predicts that equal tumour response would require a weekly fraction of ~ 60 Gy and that the weekly fractionation should be associated with a 14 per cent lower complication rate.

Thus it seems to be indicated that the Cohen model may also be used on schedules with 3 and 1 fraction per week.

At the time of writing, data from August 1972 to December 1977 are being collected (60 Gy/30 fractions/41 days) and although the follow-up on some of these patients is short, it may already be stated that it is very unlikely that addition of these data will cause a change in the working hypothesis based on the Cohen model.

SUMMARY

Long term results, obtained in the treatment of 308 patients with laryngeal carcinoma, are analysed with respect to local recurrence and complications. In this analysis the Cell Population Kinetic model proposed by COHEN (1971) is used. The model for squamous cell carcinoma is found to be useful and in close agreement with observations of local recurrence, although a slight modification may be justified from the present data. No special model exists for late edema, but the model for late effects in the vascular stroma seems to be useful in this respect. Based on the model, a working hypothesis has been proposed by which it is possible to predict the frequency of local recurrence and late edema. An optimum treatment schedule is calculated and converted to a split-course technique.

REFERENCES

- COHEN L. A cell population kinetic model for fractionated radiation therapy. *Radiology* 101 (1971) 419.
- (a) Cell population kinetics in radiation therapy. Optimization of tumor dosage. *Cancer* 1 (1973) 236.
- (b) An interactive program for standardization of prescriptions in radiation therapy. *Comput. Prog. Biomed.* 3 (1973) 27.
- (a) Dose time relationship: Computation of cell lethality following fractionated radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* 4 (1978) 267.
- (b) Derivation of cell population kinetic parameters from clinical statistical data (program RAD3). *Int. J. Radiat. Oncol. Biol. Phys.* 4 (1978) 835.
- and REDPATH J. L. Derivation of survival kinetic parameters for cell populations by computer simulation of radiobiological data. *Radiat. Res.* 100 (1977) 387.
- and SVEINSSON H. Cell population kinetics and dose time relationships for post irradiation injury of the brachial plexus in man. *Acta radiol. Oncology* 17 (1978) 161.
- DVIVEDI M. and PRADHAN D. G. Immediate results of

of the treatment period) will not affect the probability of local control if the absorbed dose is increased by approximately 10 per cent to compensate for tumour growth during the treatment interval and even then the patient will tolerate the treatment better. The opinion of these authors is based on the treatment of several thousands of patients including some with laryngeal carcinoma.

Fig. 6 illustrates how the Cohen model for GUT, VASC, SKIN and SQCA, the Kirk model (KIRK et coll. 1975) and the Ellis model (ELLIS 1968, 1969, WINSTON et coll. 1969) would predict the effect of an interval of 30 days introduced in the treatment schedule 60 Gy/30 fractions/41 days.

The Cohen model (SQCA) predicts that approximately a 10 per cent higher absorbed dose should be given in order to obtain equal probability of local control on squamous cell carcinoma. This appears to be fairly independent of the position of the interval (and of tumour size).

As for the effect in normal tissue, the Ellis model is not in accordance with the experience concerning the split course technique; the Kirk model is so to some degree, whereas the Cohen model is so to a high degree with respect to the tissues in the gastrointestinal tract (GUT) and the vascular stroma (VASC). (Rectal complications have been analysed in 664 patients with carcinoma of the uterine cervix (HJELM HANSEN & SELL 1977). Excellent agreement with the Cohen model was found, and the split course technique based on the Cohen model has been adopted since January 1978 with good results.) The Cohen model used for SKIN behaves differently and in fact (like the Ellis model) indicates that the frequency of complications increases in the split-course schedule. Except when the parameter EQD1 SKIN is used, the interval should preferably be placed in the last half of the treatment period.

The opinions based on the split course schedule mostly concern the acute reactions (patients tolerate the treatment better) while the Ellis model is based on late reactions. However, the Cohen model (VASC) is also based on late reactions (late effects in the vascular stroma such as telangiectasis or end arteritis) and the disagreement between the two models is an obvious matter for analysis.

If the calculations are based on the working hypothesis and if the size of one fraction is restricted to the usual value ($D_c=2.00$ Gy) and the treatment interval to 21 days, the optimum treat-

ment ($D_c=65$ Gy/30 fractions/41 days) converted to split course technique becomes $D_c=72$ Gy/36 fractions/70 days with an interval of 21 days inserted after 20 fractions. This schedule has been used during 1978 when the side of the equivalent square field did not exceed 8 cm. When larger fields were used, the schedule was reduced by 3 fractions.

In the high level schedule, the value of EQD1 SQCA will be greater than 22 Gy and the value of EQD1 VASC will be less than 12 Gy. Thus, according to the working hypothesis, the frequency of local recurrence (Fig. 2) should be less than 20 per cent (the overall frequency is calculated to 13%) while the frequency of late edema (Fig. 5) should be very low, since the previous experience (although without split course) is 0/67 (confidence limits 0.00–7.60%).

However, based on other parameters than EQD1 VASC, different predictions are found, indicating that the frequency of late edema may increase to as much as 30 per cent.

Preliminary experience with the split course schedule. From the experience gained during the first year (67 patients), the impression is that the patients tolerate the split course schedule in approximately the same way as the old schedule; i.e. acute effects are similar in spite of a considerable increase of absorbed dose. The patients like the rest period and most patients report that any reactions felt at the beginning of the interval disappear during its course. However, slight reactions often reappear within the first week of continued treatment.

It is too early for any statement about tumour response and late reactions. However, two surprisingly strong reactions have been observed (one required a total laryngectomy) and in order to reduce the risk it has been decided to use a reduced schedule from January 1979 (omitting the last two fractions) and await the analysis of local recurrence among the 67 patients with the high level split course schedule during 1978.

Discussion

COHEN (1978b) discusses some of the limitations and possibilities of the model and the reader is referred to this discussion.

The model (SQCA) has been tested on the present material and on 664 patients with carcinoma of the uterine cervix and although the analysis of this last material is much more complex, the same tendency

excellent agreement with the model was found. In these materials the model is predicting slightly lower frequencies of local recurrence than observed. Since the present data for high values of EQD1 (SQCA 22 Gy) would justify only a slight adjustment of the model and since this part of the response curve is the important part with respect to optimization, no adjustment will be proposed at this stage. The model has only been tested on schedules with 5 fractions per week with only a few (and small) unplanned treatment intervals.

The optimum schedule calculated in the present analysis could (according to the model) be better if the restriction of 5 fractions per week could be disregarded. Comparison with results from the literature is difficult because information about tumour size is lacking. However, at least a qualitative comparison could be made with two randomized trials comparing 5 fractions per week with 3 fractions and fraction per week respectively.

One of these is the trial run by the British Institute of Radiology since 1963 of 5 versus 3 fractions per week in a number of different schedules (Second Progress Report 1968). In the Sixth Interim Progress Report (1978) on this test it is reported that among 87 patients treated, those who received 5 fractions per week have a slightly higher (~5%) local recurrence free rate.

According to the Cohen model some of the schedules used (long overall time) would be better (5-10%) with 5 fractions per week while others would be equal or slightly better (0-3%) with 3 fractions per week. Thus it is in excellent agreement with the model that only a small difference has been observed. The largest difference has been observed among the smallest tumours (cords mobile) but it should be noted that any odd distribution of tumour size in the two groups would give rise to a much larger difference than the one caused by the fractionation. According to the model, the schedules with 5 fractions per week should give rise to more (5 to 10%) complications which however have not been observed.

The second trial of 5 versus 1 fraction per week was presented by DIVEDI *et al.* (1978) and includes 341 patients, among which 202 patients were head and neck cases. Although the follow up is incomplete and short, the authors conclude that the tumour response is equal and that 1 fraction per week is tolerated better. The schedules in question were 30x2.00 Gy/41 days and 6x6.70 Gy/36 days.

The Cohen model predicts that equal tumour response would require a weekly fraction of 6.60 Gy and that the weekly fractionation should be associated with a 14 per cent lower complication rate.

Thus it seems to be indicated that the Cohen model may also be used on schedules with 3 and 1 fraction per week.

At the time of writing, data from August 1972 to December 1977 are being collected (60 Gy/30 fractions/41 days) and although the follow up on some of these patients is short, it may already be stated that it is very unlikely that addition of these data will cause a change in the working hypothesis based on the Cohen model.

SUMMARY

Long term results obtained in the treatment of 308 patients with laryngeal carcinoma are analysed with respect to local recurrence and complications. In this analysis the Cell Population Kinetic model proposed by COHEN (1971) is used. The model for squamous cell carcinoma is found to be useful and in close agreement with observations of local recurrence, although a slight modification may be justified from the present data. No special model exists for late edema, but the model for late effects in the vascular stroma seems to be useful in this respect. Based on the model, a working hypothesis has been proposed by which it is possible to predict the frequency of local recurrence and late edema. An optimum treatment schedule is calculated and converted to a split-course technique.

REFERENCES

- COHEN L. A cell population kinetic model for fractionated radiation therapy. *Radiology* 101 (1971) 419.
- (a) Cell population kinetics in radiation therapy. Optimization of tumor dosage. *Cancer* 1 (1973) 236.
- (b) An interactive program for standardization of prescriptions in radiation therapy. *Comput. Prog. Biomed.* 3 (1973) 27.
- (a) Dose time relationship: Computation of cell lethality following fractionated radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* 4 (1978) 267.
- (b) Derivation of cell population kinetic parameters from clinical statistical data (program RAD3). *Int. J. Radiat. Oncol. Biol. Phys.* 4 (1978) 835.
- and REDPATH J. L. Derivation of survival kinetic parameters for cell populations by computer simulation of radiobiological data. *Radiat. Res.* 69 (1977) 387.
- and SVENSSON H. Cell population kinetics and dose time relationships for post irradiation injury of the brachial plexus in man. *Acta radiol. Oncology* 17 (1978) 161.
- DIVEDI M. and PRADHAN G. Immediate results of

- weekly fractionation in external radiotherapy. *Int J Radiat Oncol Biol Phys* 4 (1978) 573
- ELLIS F Time fractionation and dose rate in radiotherapy. *Front radiat Ther Oncol* 3 (1968) 131
- Dose time and fractionation. A clinical hypothesis. *Clin Radiol* 20 (1969) 1
- HJELM HANSEN M and SELL A Local recurrence and rectal complications in a material of 664 cases treated by radiotherapy of cancer in the cervix. Digest of the Ninth Nordic Meeting of Clinical Physics p 95. Gothenburg 1977
- JORGENSEN K, ANDERSEN A P and LUND C Laryngeal carcinoma. II Analysis of treatment results using the Ellis model. *Acta radiol Oncology* 18 (1979) 385
- HOLST L R Split-course radiotherapy of cancer. *Acta radiol Ther Phys Biol* 6 (1967) 313
- (a) Clinical experience with split-course radiotherapy. A randomized clinical trial. *Radiology* 92 (1969) 591
- (b) Split-course techniques. Time and dose relationship in radiation biology as applied to radiotherapy. NCI AEC Conference p 292. Brookhaven National Laboratory Report No. 50203. 1969
- Clinical experience with split-course megavoltage radiotherapy. International Congress Series No. 249 p 396. Excerpta Medica. Amsterdam 1971
- JORGENSEN K, HJELM HANSEN M, ANDERSEN A P and LUND C Laryngeal carcinoma. I Treatment results. *Acta radiol Oncology* 18 (1979) 282
- KIRK J, GRAY W M and WATSON E R Cumulative radiation effect. V Time gaps in treatment regimes. *Clin Radiol* 26 (1975) 565
- KOSKINEN M O and SPRING E Build up and build-down measurements with thin LiF teflon dosimeters with special reference to radiotherapy of carcinoma of the larynx. *Strahlentherapie* 145 (1973) 565
- LUND C, JORGENSEN K, HJELM HANSEN M and ANDERSEN A P Laryngeal carcinoma. III Treatment results in relation to microscopic score. *Acta radiol Oncology* 18 (1979) 497
- PETO R, PIKE M C, ARMITAGE P, BRESLOW N E, COOPER D H, HOWARD S V, MANTEL N, MCPHERSON M, PETO J and SMITH P G Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II Analysis and examples. *Brit J Cancer* 35 (1977) 1
- SACHER G A and TRUCCO E Theory of radiation injury and recovery in self renewing cell populations. *Radiat Res* 29 (1966) 236
- SAMBROOK II K Clinical trial of a modified (split-course) technique of X ray therapy in malignant tumours. *Clin Radiol* 13 (1962) 1
- (a) Theoretical aspects of dose time factors in radiotherapy technique. I Dose factors. *Clin Radiol* 13 (1963) 290
- (b) Theoretical aspects of dose time factors in radiotherapy technique. II Time factors. *Clin Radiol* 14 (1963) 433
- SCANLON P W Initial experience with split-course periodic radiation therapy. *Amer J Roentgenol* 83 (1960) 632
- Split-course radiotherapy for head and neck cancer. *Front radiat Ther Oncol* 3 (1968) 195
- Second progress report of the British Institute of Radiology fractionation working party. 1968. *Brit J Radiol* 41 (1968) 723
- Sixth interim progress report of the British Institute of Radiology fractionation study of 3F/week versus 5F/week in radiotherapy of the laryngo-pharynx. *Brit J Radiol* 51 (1978) 241
- UICC TNM classification of malignant tumours. Union International Contre le Cancer. Geneva 1979
- WINSTON B M, ELLIS F and HALL E J The O₁₀/TD₅₀ NSD calculator for clinical use. *Clin Radiol* 13 (1969) 8

COMPLICATIONS FROM IRRADIATION OF CARCINOMA OF THE UTERINE CERVIX

J. ALERT, J. JIMENEZ, L. BELDARRAIN, J. MONTALVO and C. ROCA

Cervical carcinoma is the second most frequent malignant neoplasm in Cuban women with an annual average of about 650 new patients and a crude incidence rate of 16.1 per 100 000 and year (Registro Nacional del Cáncer 1975). Irradiation is the principal method used with a combination of intracavitary and external irradiation. Its use implies a certain risk of complications from organs situated within the irradiated volume, the intestine and bladder being the organs most often affected. The types and the frequency of complications in a group of patients treated by irradiation alone are now reported.

Material and Methods

The material consisted of 2248 patients with carcinoma of the uterine cervix who were irradiated between 1966 and 1972 at three oncologic centers: 1280 (56.9%) at Instituto de Oncología (Havana City), 803 (35.8%) at Hospital Oncológico Docente (Santiago de Cuba) and 165 (7.3%) at Hospital Oncológico (Camaguey). The method of treatment and the results have been reported previously (JIMENEZ et al. 1979).

Complications were classified as early when the symptoms and signs appeared during the irradiation series or within 6 months from its completion and as late when they appeared 6 months or later after the end of irradiation. Cases where malignant tissue was found in the walls of fistulas were excluded.

Results

Complications were recorded in 458 patients (20.4%). The total number was 523 as some patients had more than one complication (Table). Of these 305 (58.3%) were classified as early and 218 (41.7%) as late.

Proctitis was the most frequent complication and occurred in 15.5 per cent of the total number of patients. The degree of proctitis varied from only tenesmus and edema of the rectal wall to ulceration and bleeding. During or at the end of irradiation some patients developed acute symptoms such as tenesmus, painful defecation and diarrhea which were classified as early proctitis. Late proctitis usually appeared between six months and one year from the end of irradiation. In 176 patients late proctitis occurred and in all but 10 this was preceded by early proctitis.

More severe complications as fistulas, ureteral stenosis with hydronephrosis, enterocolitis, sigmoiditis, intestinal obstruction and skin necrosis occurred in 81 patients (3.6%). Fistulas developed only in 36 patients (1.6%).

Colostomy was performed in 11 patients (0.5%) for the following reasons: severe proctitis with ulceration of the rectal wall and uncontrollable bleeding (3 patients), fistulas (3 patients) and intestinal obstruction (5 patients).

A similar incidence of complications occurred af

Table

Type and frequency of complications following irradiation

Type of complication	Number	Per cent
Proctitis	348	66.5
Cystitis	74	14.1
Recto-vaginal fistula	28	5.3
Ureteral stenosis with hydronephrosis	24	4.6
Panniculitis	17	3.3
Intestinal obstruction	13	2.5
Vesico-vaginal fistula	8	1.5
Sigmoiditis	4	0.8
Edema of legs	3	0.6
Skin necrosis	2	0.4
Enterocolitis	1	0.2
Fistula between sigmoid and jejunum	1	0.2

ter the two principal techniques used: external irradiation followed by intracavitary radium applications or intracavitary radium applications followed by external irradiation.

Discussion

Radiation therapy, similar to surgery or cytostatic therapy, implies certain risks of complication. The literature reporting and discussing the incidence and types of complications is extensive (GRAY & KOTTMEIER 1957, FLETCHER et coll 1958, CHAU et coll 1962, CASTRO et coll 1970, STROCKBINE et coll 1970, BORONOW & RUTLEDGE 1971, FLETCHER 1971, JOELSSON et coll 1971, SLATER & FLETCHER 1971, MICKAL et coll 1972, ROSWIT et coll 1972, VILLASANTA 1972, FRIBERG & JOHNSON 1974, MARUYAMA et coll 1974, CHISM et coll 1975, EINHORN 1975, JOHNSON 1976, MARCIAL 1977, PEREZ et coll 1977, ALERT 1978, ALERT et coll 1978). The complications are caused by the radiation dose to different parts of the small pelvis and the lower part of the abdomen, which causes interstitial edema, necrosis and local fibrosis. Individual variations in sensitivity and the pretreatment condition (general condition, local inflammatory diseases) certainly also play a role.

In practice, the distribution of the dose can deviate considerably from the calculated ideal dosimetry due to different clinical factors distorting the geometry of the small pelvis (SCHWARZ 1969). The determination of the doses at points in the paracervical region with regard to the intracavitary

radium arrangement can be a dangerous practice. Both with inverted T and the linear arrangement, the radiation sources decrease in the dose measured from the surface of the applicators to the periphery of the small pelvis. This decrease is influenced by different factors such as the distance between the vaginal sources, the length of the linear arrangement, etc. (FLETCHER, ALERT). Vaginal applicators situated preferentially in the posterior vault increase the dose in the rectovaginal wall and increase the risk of proctitis.

Although 348 patients had proctitis, colostomy was needed only in 3. Rectovaginal fistulas occurred in 28 patients, an incidence of 1.2 per cent.

External irradiation before radium application causes high doses to large parts of the intestine. By the external irradiation, the thickness of the uterine wall may also be reduced, which may cause a higher intestinal dose from the intracavitary irradiation. It seems likely that both these factors contribute to the intestinal complications found (EINHORN).

Sigmoiditis has been reported as a specific complication of whole pelvis irradiation, especially after external irradiation alone (FLETCHER). The patients with a fistula between the sigmoid and jejunum in the present series had received external and intracavitary irradiation. Probably the uterine fundus was situated close to the sigmoid, with a resulting high local dose.

Ureteral structures, which have been reported to occur with a very low incidence (SLATER & FLETCHER), were observed in 24 patients. The signs of obstruction varied from slight or moderate dilatation of the ureter and renal pelvis to complete lack of kidney function on the obstructed side.

The total observed incidence of severe complications was in the present series 3.5 per cent. Only 0.5 per cent of the patients was surgical treatment of complications required.

The risk of complications in connection with radiation therapy may be reduced to some extent by careful dose planning and continuous observation of the patient during the treatment. However, it must be emphasized that certain frequency of complications must always occur as a consequence of irradiation.

SUMMARY

The types and frequency of complications in 348 patients irradiated for carcinoma of the cervix using different techniques are reported. Proctitis was the most frequent

complication Severe complications such as fistulas ureteral stenosis with hydronephrosis enterocolitis sigmoiditis intestinal obstruction and skin necrosis occurred in 81 patients (3.6%). Only 36 patients (1.6%) developed fistulas

REFERENCES

- ALERT J Las complicaciones de los tratamientos radiantes de los carcinomas cervicouterinos Experiencias en 626 pacientes (In Spanish) *Rev. cuba. Med.* 17 (1978) 303
- JIMENEZ M J y RODRIGUEZ M J Las complicaciones intestinales en los tratamientos por radiaciones ionizantes de los carcinomas del cuello del útero Estudio en una serie de 1530 pacientes (In Spanish) *Rev. cuba. Méd.* 17 (1978) 423
- BORONOW R C and RUTLEDGE F N Vesicovaginal fistula radiation and gynecologic cancer *Amer. J. Obstet. Gynec.* 111 (1971) 85
- CASTRO J R, ISSA P and FLETCHER G Carcinoma of the cervix treated by external irradiation alone *Radiology* 295 (1970) 163
- CHAU P M, FLETCHER G H, RUTLEDGE F N and DODD H Complications in high dose whole pelvis irradiation in female pelvic cancer *Amer. J. Roentgenol.* 87 (1962) 22
- CHISM S E, KEYS H M and GELLIN M T Carcinoma of the cervix A time-dose analysis of control and complications *Amer. J. Roentgenol.* 123 (1975) 84
- EINHORN N Frequency of severe complications after radiation therapy for cervical carcinoma *Acta radiol. Ther. Phys. Biol.* 14 (1975) 42
- FLETCHER G H Cancer of the uterine cervix *Janeway Lecture* 1970 *Amer. J. Roentgenol.* 111 (1971) 225
- BROWN T C and RUTLEDGE F N Clinical significance of rectal and bladder dose measurements in radiation therapy of cancer of the uterine cervix *Amer. J. Roentgenol.* 79 (1958) 421
- FRIEBERG L and JOHNSON J E Bladder and intestinal injuries following intracavitary irradiation of carcinoma of the uterine cervix *Acta radiol. Ther. Phys. Biol.* 13 (1974) 288
- GRAY M J and KOTTMEIER H Rectal and bladder injuries following radium therapy for carcinoma of the cervix at the Radiumhemmet *Amer. J. Obstet. Gynec.* 74 (1957) 1294
- JIMENEZ J, ALERT J, BELDARRAIN L, MONTALVO J and ROCA C Carcinoma of the uterine cervix Results of treatment in 2248 cases *Acta radiol. Oncology* 18 (1979) 465
- JOELSSON I, RÄFL. and SÖDERBERG H Stenosis of the small bowel as a complication in radiation therapy of carcinoma of the uterine cervix *Acta radiol. Ther. Phys. Biol.* 10 (1971) 593
- JOHNSON J E Bladder and intestinal injuries following radiation therapy of carcinoma of the uterine cervix *Acta radiol. Ther. Phys. Biol.* 15 (1976) 541
- MARCIAL V Carcinoma of the cervix Present status and future *Cancer* 39 (1977) 945
- MARUYAMA Y, VAN NAZELL J R, UTLEY J, VIDER M and PARKER J C Radiation and small bowel complications in cervical carcinoma therapy *Radiology* 112 (1974) 699
- MICKAL A, TORRES J H and SCHLOSSER J V Complications of therapy for carcinoma of the cervix *Amer. J. Obstet. Gynec.* 112 (1972) 556
- PEREZ C A, ZIVNIESKA F, ASKIN F, CAMEL M, RAGAN D and POWERS W E Mechanisms of failure in patients with carcinoma of the uterine cervix extending into endometrium *Int. J. Radiat. Oncol. Biol. Phys.* 2 (1977) 651
- ROSWIT B, MALSKEY S J and REID C H Severe radiation injuries of the stomach, small intestine, colon and rectum *Amer. J. Roentgenol.* 114 (1972) 460
- SCHWARZ G Evaluation of Manchester system of treatment of carcinoma of the cervix *Amer. J. Roentgenol.* 105 (1969) 579
- SLATER J M and FLETCHER G H Ureteral strictures after radiation therapy for carcinoma of the uterine cervix *Amer. J. Roentgenol.* 111 (1971) 269
- STROCKBINE M F, HANCOCK J E and FLETCHER G H Complications in 831 patients with squamous cell carcinoma of the intact uterine cervix treated with 3000 rads or more whole pelvis irradiation *Amer. J. Roentgenol.* 108 (1970) 293
- VILLASANTA V Complications of radiotherapy for carcinoma of the uterine cervix *Amer. J. Obstet. Gynec.* 114 (1972) 717

CHROMOSOME COUNTS OF ^{90}Sr INDUCED OSTEOSARCOMAS IN MICE

I Transplanted tumour series

H BERGMAN and A NILSSON

Hypotheses concerning radiation induced malignant tumours which for the present are consonant with available data are those concerning initiating and promoting events in a multi step mechanism (NILSSON 1975) supposed to be somatic mutations. As the present knowledge of chromosome injury is limited and as many radionuclides are extremely potent carcinogens, cytogenetic investigations should contribute to an increased understanding of the induction mechanism. For this purpose ^{90}Sr induced tumours should constitute a suitable experimental model as the development of these tumours (NILSSON 1966) their histologic type (NILSSON 1962) dose dependence (NILSSON 1969) and several other characteristics have been clarified in detail. The main purpose of the present investigation was to form a conception of the chromosome numbers of the primary tumours and especially the late chromosomal progression by serial *in vivo* transplantation. An intention was also to analyse whether additional and predetermined steps could be detected as found in serially transplanted Rous rat sarcomas (MITELMAN 1972). A comparison was made between the chromosome pattern of fast and slow growing tumour series (Parts II and IV BERGMAN 1980) and whether transplantation to mice with a changed immunologic status is combined with a modified chromosome distribution (Part II). A comparison was also performed on serially *in vivo* transplanted tumours and parallelly *in vitro* cultured tumour cells (Part III BERGMAN 1980). The chromosome pattern of early ^{90}Sr induced primary tumours so called tumour buds will later be investigated in order to ascertain whether chromosome aberrations can be proved at these relatively early stages of the tumour evolution.

Material and Methods

Inbred about 60-day old female mice of the CBA strain were used. Primary osteosarcomas were induced by intravenous injection of 14.8 to 29.6 kBq $^{90}\text{Sr}(\text{NO}_3)_2$ per g body weight (Table 1). Tumours were localised by radiography 288 to 403 days after the injection of ^{90}Sr (Fig. 1). Tumour bearing mice were killed by cervical dislocation. Peripheral and homogeneous parts of the tumours were placed in physiologic saline and pinhead sized pieces were subcutaneously transplanted to the neck of 10 male mice. From these mice a succeeding generation was obtained by transplantation from one of the outgrown tumours to another 10 mice. A diagram of the transplantations is presented in Fig. 2. By this type of serial transplantation 3 transfer series A to C were established from 3 primary tumours. The number of transfer generations and tumours examined is given in Tables 2, 4 and 5. Samples were taken from the primary tumours as well as from the tumours used for transplantation for chromosome analysis. After an outgrowth period of 10 to 72 days the tumour volume was calculated by using a caliper. The tumours were carefully dissected and all necrotic parts were discarded. Small pieces of the tumours were prepared by mincing through a steel wire gauze. The material was incubated at 38°C in a 1% sodium-citrate solution for 15 min and centrifuged at 800 rpm for 10 min. The supernatant was pipetted off and the cell suspension was fixed in glacial acetic acid and ethanol (1:3) for 30 min. The fixation procedure was repeated 2 to 3 times. The preparations were air dried and the cells

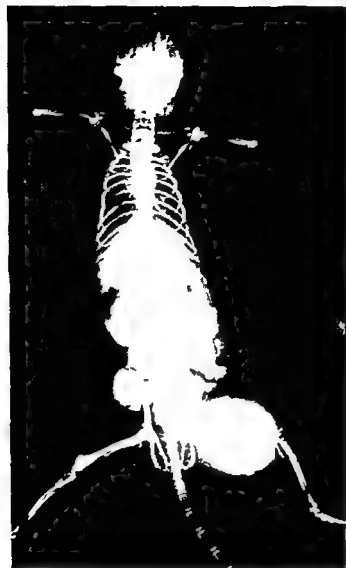


Fig. 1 Primary osteosarcoma A in the left femur 288 days after the injection of ^{90}Sr

stained in acetic orcein (2% orcein in 60% acetic acid) for 1 h or according to the conventional Giemsa method. The preparations were observed in a Wild Heerburg M 20 microscope. Cells suitable for chromosome counting were selected at a magnifica-

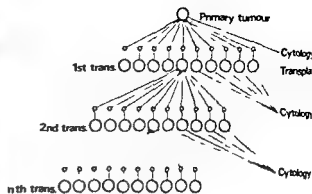


Fig. 2 Diagram of transplantations

tion of $200\times$ and analysed at $1000\times$. Only apparently well spread and unbroken metaphases were chosen. At an early stage an optimum number of well spread metaphases per tumour were examined. However due to poor preparations it later became necessary to decrease the number of cells to 25, even below this level. At the most 5 tumours per transfer generation were analysed, but only transfers from which it was possible to analyse at least 2 tumours are presented. Due to the few chromosome counts per tumour it was preferred to refer to the chromosome counts per transfer generation. It should also be noted that from transfer generation 25 and on analysis was performed only sporadically.

For histologic examination sections of the primary tumours and a selection of tumours along the transfer series were fixed in Stevie's solution, embedded and stained according to the van Gieson method or with haematoxylin-eosin.

Definition of tumour classification: The osteosarcomas were subdivided into fibroblastic, chondroblastic, osteoblastic or osteoclastic types. In addition pleomorphic, mixed and anaplastic types were distinguished depending upon whether a strong pleomorphic cell type equal parts of at least three

Table 1
Different characteristics of the primary osteosarcomas

Primary tumour	Dose ^{90}Sr (kBq/g body weight)	Induction time (days)	Site of the tumour in the skeleton	No. of cells analysed	Chromosome number											
					35	36	37	38	39	40	41	42	61	72	73	74
A	29.6	288	L. femur	26	1	1	1	10	7	3		1				
B	14.8	403	L. tibia-femur	-												
C	14.8	403	■ femur	17					2	III	3	1	1			

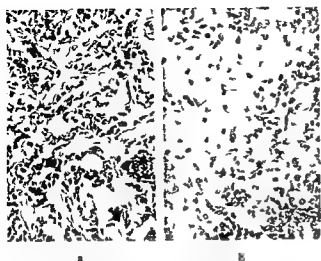
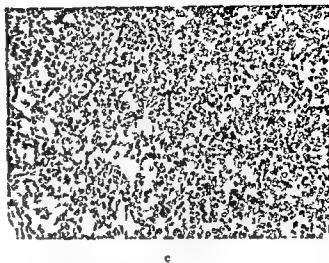


Fig. 3 Primary osteosarcoma B from a mouse given 14.8 kBq ⁹⁰Sr/g body weight appearing 403 days after the injection. Dis-
unct bone (a) and cartilage (b) formation but with predominance



of low differentiated and pleomorphic mainly fibroblastic tissue
(c) infiltrating surrounding muscles. Abundance of mitosis
Haematoxylin eosin $\times 240$

cell components or a low differentiated tissue pre-
dominated. This subdivision has been recommended
by the Committee of Pathology of the European
Late Effects Project Group (EULEP 1971).

Results

Histology. The primary tumour A was character-
ized as a predominantly fibroblastic osteosarcoma
with a slight osteoid formation. Primary tumour B

Table 2

Transfer series A. Different characteristics of the transfer generations examined

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm ³)	Mean growth per day (cm)	Pre dominating chromo- some No	Per cent	Percentage of cells with metacentrics
2	5	108	19.2	0.88	0.046	38	67.1	5.2
3	5	164	17.0	1.00	0.059	38	51.2	6.0
4	4	173	18.8	1.03	0.055	38	50.9	5.9
5	9	208	18.1	1.16	0.064	38	36.5	4.6
6	3	111	21.3	0.89	0.042	37	40.0	5.9
7	6	272	24.3	1.03	0.042	37	47.4	3.9
8	3	137	24.3	0.73	0.030	37	43.2	9.8
9	4	101	19.5	0.94	0.048	37	59.4	5.9
10	3	75	20.3	1.07	0.053	37	48.0	10.0
17	6	150	16.5	0.79	0.048	37	31.3	5.3
11	2	50	6.0	1.49	0.057	37	48.3	16.0
14	4	95	20.5	0.88	0.043	37	48.4	0
16	3	75	19.0	1.12	0.059	37	34.7	2.7
17	7	161	19.9	1.03	0.052	37	36.0	3.1
18	3	75	15.3	0.87	0.057	40	84.0	0
23	3	75	14.7	0.95	0.065	40	90.7	0
43	3	75	15.0	0.75	0.050	40	66.7	1.3
45	3	75	15.7	0.84	0.054	40	41.3	1.3
48	4	100	9.8	0.35	0.036	40	71.0	1.0
50	3	75	14.0	1.56	0.111	40	46.7	0
53	3	75	11.3	0.66	0.058	40	66.7	0
54	5	125	16.0	0.99	0.062	40	64.0	13.5
55	4	100	15.0	0.79	0.053	40	70.0	14.0

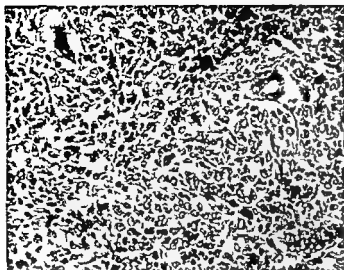


Fig 4

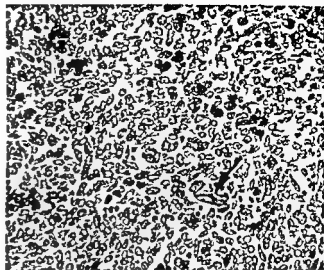


Fig 3a

Fig 4 From transfer series A. Highly anaplastic tumour from the 22nd transfer generation: numerous giant cells: van Gieson $\times 240$.

Fig 5 a) From transfer series C. Highly anaplastic osteosarcoma from a tumour of transfer generation 2. Tumour bone (\rightarrow): van Gieson $\times 240$. b) Tumour from transfer generation III. Area with abundance of giant cells and tumour bone: van Gieson $\times 240$.

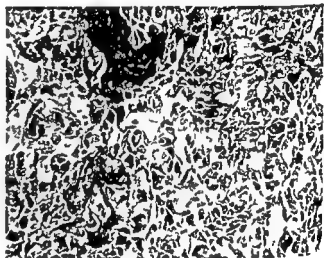


Fig 3b

Table 3

Transfer series A. The percentile chromosome distribution of 11 transfer generations

Transfer generation	No of tumours examined	No of cells counted	Chromosome number															
			32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
2	4	108		0.9			1.9	8.3	61.1	13.0	3.7	1.9		0.9				
5	9	98	0.5		3.4	1.9	1.5	79.8	36.5	7.2	0.5	1.4	0.5		0.5			0.5
6	3	85	1.2		2.4	4.7	11.8	40.0	15.3	15.3	1.7		1.7					
9	4	101				2.0	17.9	59.4	11.9	2.0	3.9					1.0		
17	7	161			1.9	7.4	8.1	36.0	8.7	4.9	24.8	0.6						
25	3	75						1.3		2.7	90.7	7.7						
43	3	75									66.7		1.3					
45	3	75									41.3		1.3					1.1
49	3	75									46.7		1.3		1.3			1.1
44	4	174							0.8	0.8	64.0					0.8		
45	4	100								3.0	70.0		1.0					

Table 4

Transfer series B. Different characteristics of the transfer generations

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm ²)	Mean growth per day (cm ²)	Pre dominating chromosome No	Per cent	Percentage of cells with metacentrics
1	5	125	20.2	0.79	0.039	40	65.6	0
2	5	125	25.0	1.43	0.057	40	25.6	37.0
3	5	125	18.0	0.93	0.057	60	50.4	5.6
4	5	125	17.7	1.14	0.064	40	51.2	0.8
5	5	125	18.6	1.42	0.076	40	33.6	2.4
6	5	125	13.8	1.03	0.075	40	73.6	0.8
7	5	125	14.0	1.26	0.090	40	39.2	7.4
8	5	125	14.2	1.72	0.121	40	55.2	0
9	5	125	13.0	1.41	0.108	40	50.4	1.6
10	5	125	13.0	1.38	0.106	40	63.2	0
11	5	125	15.4	0.84	0.055	40	86.4	0
12	5	125	14.6	1.50	0.103	40	72.8	0.8
13	5	125	16.0	1.23	0.077	40	67.2	0
14	5	125	13.4	0.93	0.069	40	56.0	0
15	4	100	14.3	1.06	0.074	40	41.0	0
16	4	99	15.8	1.24	0.078	61	40.4	0
17	5	175	15.0	1.06	0.071	40	36.0	0.8
18	5	125	14.4	0.73	0.051	40	52.0	0
19	3	75	18.7	0.83	0.044	60	36.0	1.3
20	5	125	16.0	1.05	0.066	40	48.0	0.8
21	5	125	16.6	1.70	0.072	40	60.0	0
22	4	100	15.3	1.29	0.084	40	56.0	0
23	5	125	17.0	0.94	0.078	40	59.2	0.8
25	3	75	14.3	1.09	0.076	40	61.3	0
34	4	100	13.0	0.75	0.058	40	62.0	0

Table 5

Transfer series B. The percentile chromosome distribution of 10 transfer generations

Transfer generation	No of tumours examined	No of cells counted	Chromosome number																																			
			39	40	41	42	43	53	54	55	56	57	58	59	60	61	62	63	64	65																		
1	5	125	65.6 1.6																																			
3	5	125	2.4	22.4	0.8												16	16	32	2.4	40	5.6	88.48	0.8														
5	5	125	73.6																32	6.4	6.4	50.4	4.8	16	1.6													
7	5	125	39.2																0.8 1.0 1.8 0.8																			
11	5	125	0.8	86.4	1.6	0.8												0.8	2.4	8.0	16.8	7.6	88.7.4															
16	4	99	1.0	16.2	0.8												2.0	1.0	4.0	24.2	40.4	10.1	1.0															
19	3	75	18.7 1.3																5.3	5.3	25.3	36.0	5.3	2.7														
20	5	125	48.0																1.6												16	8.0	33.6	4.8	0.8	1.6		
25	3	75	1.3	61.3	1.3												1.3	1.3	6.7	6.7	13.3	6.7																
34	4	100	62.0																1.0	1.0	4.0	20.0	10.0	1.0														

examined with regard to chromosome pattern in 55 transfer generations. The percentile chromosome distribution from 11 selected generations is presented in Table 3. The chromosome counts of the primary tumour are given in Table 1 and different

characteristics of the transfer generations in Table 2. The primary tumours as well as the early transfer generation (2-4) were mainly characterized by a predominance of 38 chromosome cells (Fig. 6) but often with a wide range of variation. With the fifth

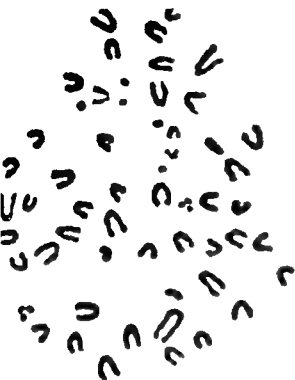


Fig. 8 A 56-chromosome cell from a tumour of transfer series B generation 4

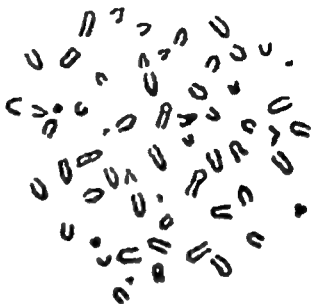


Fig. 9 A 60-chromosome cell from a tumour of transfer series B generation 4

Table 6

Transfer series C. Different characteristics of the transfer generations examined

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm ³)	Mean growth per day (cm ³)	Pre dominating chromo some No	Per cent	Percentage of cells with metacentrics
2	5	125	36.0	0.47	0.013	40	97.6	0.8
3	5	173	24.4	1.33	0.055	40	94.3	0.8
4	7	50	32.0	0.71	0.022	40	98.0	0
5	5	125	74.0	0.66	0.078	40	95.2	1.6
6	5	172	30.4	1.14	0.038	40	91.0	3.3
7	5	124	70.8	0.84	0.040	40	89.5	0
8	4	96	18.0	0.80	0.044	40	98.3	75.0
9	2	50	20.0	0.60	0.030	40	96.0	0
10	3	75	13.0	0.78	0.060	40	100.0	0
11	7	50	31.0	0.60	0.019	40	96.0	2.0
12	3	75	23.7	0.88	0.037	40	98.7	0
13	3	75	17.0	1.04	0.061	40	100.0	0
14	3	75	6.0	0.68	0.06	40	90.7	5.3
15	5	125	17.4	0.86	0.049	40	72.0	16.2
16	5	125	70.11	1.14	0.057	40	78.4	7.0
17	4	100	5.3	0.67	0.044	40	47.0	0
18	5	125	1.6	1.30	0.103	40	64.0	0
19	3	75	9.3	0.68	0.073	40	92.0	0
20	3	75	11.0	0.86	0.078	40	78.7	0

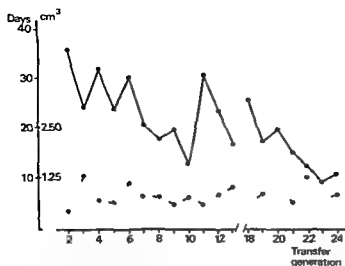


Fig. 10. Solid curve: Variation in mean outgrowth period of the tumours per transfer generation. Interrupted line: Mean size of the tumours per transfer generation. The example is from the C series.

transfer generation an increased number of 37 chromosome cells (Fig. 7) appeared. This predominance of 37 chromosome cells was persistent from generation 6 to 17. Well spread preparations were now not obtained until transfer generation 20. However, from there up to the 25th generation a modified chromosome pattern with a majority of 40 chromosome cells and only small numbers of 37- and 38-chromosome cells was found. Beyond this generation, tumour analysis was only sporadically performed, which means that the development of the chromosome pattern in the successive generations is uncertain. An analysis of the tumours of transfer generation 43 revealed a disparate chromosome distribution. Here the 40-chromosome cells were still predominant but in addition cells with chromo-

some numbers ranging from 57 to 61 were found. Except for percentile variations it was found that the split chromosome distribution was maintained also in transfer generations 45, 48, 50 and 53. In generations 54 and 55 a shift from 60 to 61 chromosome cells to cells with chromosome numbers varying from 50 to 55 but still with a majority of 40-chromosome cells.

Transfer series B. In this series 34 transfer generations were established. Chromosome counts were obtained from 117 tumours. The chromosome distributions of 10 transfer generations are presented in Table 5, while Table 4 shows different characteristics of the transfer generations examined. Except for generations 2, 16 and 17, these series show a predominance of 40 chromosome cells plus occasionally an altered chromosomal pattern within the triploid region. The percentage of 40-chromosome cells varied within the series from 16.2 to 86.4% in the last examined transfer generations showing 60 per cent of 40-chromosome cells. Concerning the triploid region, the early transfer generations displayed a wide distribution of chromosome numbers ranging from 54 to 65, but with a predominance of 60, 62 and 63-chromosome cells. Figure 8 shows a 56 chromosome cell and Figure 9 a 61 chromosome cell from a tumour of transfer generation 4. The subsequent transfer generations showed a range of 57 to 64 and a noticeably higher frequency of 60 to 64-chromosome cells was observed at 61 and 62. Transfer generations 10 and 11 displayed a preponderant number of 60- and 61-chromosome cells while transfer generations 12 to 25 were characterized by a preponderance of 40-chromosome cells but also by an increased number

Table 7

Transfer series C. The percentile chromosome distribution of 9 transfer generations

Transfer generation	No of tumours examined	No of cells counted	Chromosome number															
			39	40	41	42	47	50	51	57	53	54	55	56	57	58	59	
2	5	125	16	97.6														
5	5	125	2.4	95.2								0.8		1.6				
8	4	96	1.0	58.3														
10	3	75		100.0														
13	3	75		100.0														
19	5	15		77.0		0.8	0.8	0.8	0.8	4.0	13.6	4.8	0.8					
20	5	125	3.2	78.4						4.0	11.2	1.6	0.8					
22	5	125	2.4	64.0	1.6				0.8					0.8		1.6	4.8	
4	3	75		78.7									1.3	1.3	4.0	10.7	7.7	

No of 40 chromosome cells

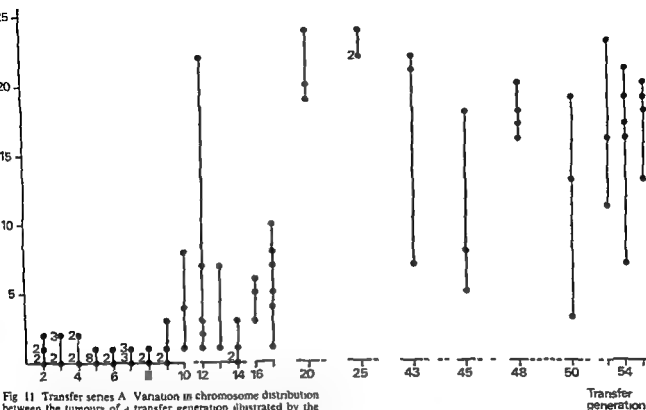


Fig 11 Transfer series A Variation in chromosome distribution between the tumours of a transfer generation illustrated by the number of 40-chromosome cells per tumour (●). The figures at the tumour symbol indicate that two or more tumours had the same number of 40-chromosome cells

of cells within the 57 to 59 region. Transfer generation 34, which was the last examined generation, displayed within the triploid region a majority of 58 chromosome cells and a range of 55 to 60.

Transfer series C In transfer series C the primary tumour and 72 tumours of 24 transfer generations were examined. Table 7 shows the percentile chromosome distribution of 9 transfer generations.

Table 7 (cont.)

1	6	63	64	65	66	69	80
						0.8	
63	73	83	135	71	21		
							0.8
0.8							
1	40	0.8					0.8
13							

The few chromosome counts obtained from the primary tumour are presented in Table 1 and differ from the characteristics of the transfer generations examined in Table 6. The primary tumour showed a narrow range of variation around 40. Except for transfer generation 8, the tumours of generations 1 to 18 contained a predominance of 40-chromosome cells ranging from 89.5 to 100 per cent. In transfer generations 19 to 24 a lower percentage of 40-chromosome cells was recorded and instead a major range of 50 to 55 and 55 to 63 for the generations 19 to 20 and 21 to 24 respectively. In this context it should also be mentioned that a large number of consecutive transplantations of tumours generally led to an increased rate of growth of the transplanted tumours (Fig 10). Furthermore the variation in chromosome distribution between tumours of one and the same transfer generation is illustrated by the number of 40-chromosome cells per tumour and generation in Figs 11 to 13.

Metacentric chromosomes The staining method used does not permit a detailed and adequate analysis of the karyotypes. However, the technique

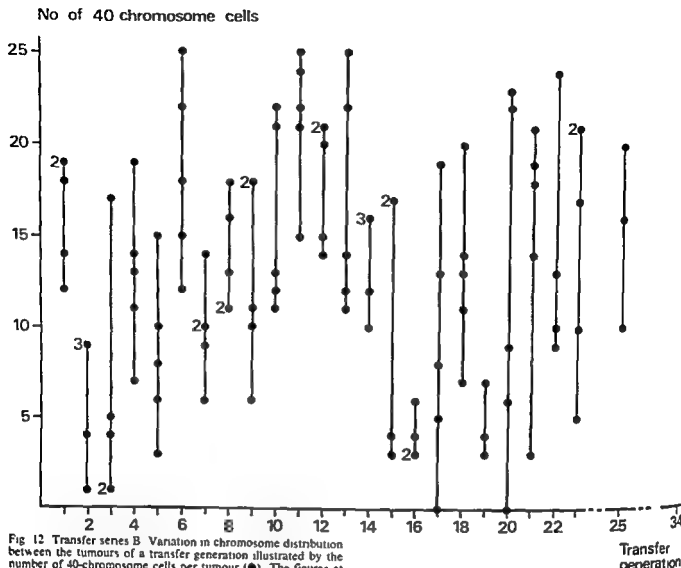


Fig. 12. Transfer series B. Variation in chromosome distribution between the tumours of a transfer generation illustrated by the number of 40-chromosome cells per tumour (●). The figures at the tumour symbol indicate that two or more tumours had the same number of 40-chromosome cells.

has revealed evidence of chromosome breakage and reunion through observation of extremely long chromosomes, minutes and metacentrics. The majority of these gross structural abnormalities appeared quite temporarily. As mouse chromosomes are normally telocentric, metacentric configurations are easily recognized. The percentage of cells with one or more metacentrics per transfer generation appear in Tables 2, 4 and 6. Even if these aberrations as well occurred quite randomly and none seemed particularly well adapted, they occasionally constituted recurrent events.

Conclusions

Whether an unbalanced karyotype may be causally related to malignancy is a basic problem that

chromosome analyses on tumours are attempting to solve. As numerous radionuclides are extremely potent carcinogens, cytogenetic investigations of radiation induced malignant tumours should contribute to an increased understanding of the induction mechanism. However, the primary intention of the present investigation of ^{90}Sr induced osteosarcoma was to obtain a conception of the chromosome pattern in late phases of the tumour progression and to examine whether additional or pre-determined steps could be detected. This was made possible by serial transplantation from the three primary tumours used. Concerning the early transfer generation, they displayed different chromosome appearance (Tables 3, 5 and 7). Thus, it was found that the A series was characterized by a predominance of number of 38- and 37-chromosome cells, the B-

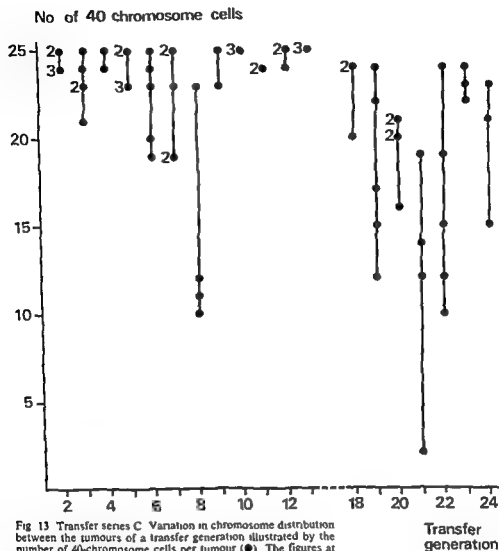


Fig 13 Transfer series C Variation in chromosome distribution between the tumours of a transfer generation illustrated by the number of 40-chromosome cells per tumour (●). The figures at the tumour symbol indicate that two or more tumours had the same number of 40-chromosome cells

series by 40 chromosome cells in combination with cells within the 52 to 64 region while the tumours of the C series displayed a distinct dominance of 40-chromosome cells. Of special interest was that in spite of this heterogeneous development striking similarities appeared but during different stages of the tumour evolution. These similarities in chromosome pattern between the three transfer series consisted of a predominant number of 40 chromosome cells in combination with cells within the 52 to 64 region. This chromosome distribution in the B series was observed already in the first transfer generation and with minor exceptions was maintained along the whole series. In the C series this type was not established until transfer generation 18 and in the A series not until transfer generation 43. However when this special distribution appeared and

except for a certain variation from tumour to tumour it seemed well balanced.

SUMMARY

From ^{90}Sr induced primary tumours three transfer series were established by serial in vivo transplantation. Chromosome counts were obtained from 2 of the primary tumours and 284 transplanted tumours. The recording of abnormalities was limited to numerical chromosome deviations and the occurrence of metacentric configurations. By means of the serial tumour transplantation the numerical chromosome progression was also analysed. Though appearing at different stages of the tumour evolution similarities in chromosome pattern were observed.

ACKNOWLEDGEMENT

The skilful technical assistance by Mrs Siw Siljerud is greatly acknowledged.

REFERENCES

- BERGMAN H. Chromosome counts of ^{90}Sr induced osteosarcomas in mice. II. Variation of the chromosome counts of slow and fast growing tumours in hyper- and nonhyperimmunized hosts. To be published in *Acta radiol. Oncology*.
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice. III. Variation of the chromosome counts of in vivo transplanted tumours, in vitro cultures and retransplanted cultured cells. To be published in *Acta radiol. Oncology*.
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice. IV. Variation of the chromosome counts when using tumours of predetermined age for transplantation. To be published in *Acta radiol. Oncology*.
- MITELMAN F. Predetermined sequential chromosomal changes in serial transplantation of Rous rat sarcoma. *Acta path. microbiol. scand. A* 80 (1972) 313.
- NILSSON A. Histogenesis of ^{90}Sr induced osteosarcomas. *Acta vet. scand.* 3 (1962) 185.
- Early development of transplanted ^{90}Sr induced osteosarcoma buds. *Acta radiol. Ther. Phys. B* (1966) 7.
- Dose dependent carcinogenic effect of radiostrontium. In: Proceedings of a symposium on radiation in cancer, organized by the International Atomic Energy Agency, p. 173. IAEA STJ/PUB/228, Vienna 1974.
- The hazard of late effects from incorporated radionuclides. In: Symposium on tumourigenic and genetic effects of radiation, p. 19. Edited by G. Walinder. Tryck Stockholm 1975.

FROM THE DEPARTMENT OF RADIATION BIOLOGY SWEDISH RESEARCH INSTITUTE OF NATIONAL DEFENCE
1704 SUNDBYBERG AND THE DEPARTMENT OF PATHOLOGY FACULTY OF VETERINARY MEDICINE
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES ■ 75007 UPPSALA SWEDEN

EFFECT OF SYNGENEIC BONE MARROW AND THYMUS CELL TRANSPLANTATION TO ^{90}Sr IRRADIATED MICE

A NILSSON P BIERKE and A BROOMÉ KARLSSON

It is a wellknown fact that the acute haematologic crisis induced by supralethal irradiation doses may be restored by transplantation of allogeneic or syngeneic bone marrow cells whereas syngeneic bone marrow cells are a necessity when midlethal irradiation doses are used. These problems have been analyzed in several reports as regards external whole body irradiations (JACOBSON *et coll* 1951, MOLE *et coll* 1955, CONGDON *et coll* 1957, URSO & CONGDON 1957, UNSGAARD 1961, DUPLAN *et coll* 1962, BARNES *et coll* 1966, JÄRPLID 1968, COVELLI *et coll* 1974, METALLI *et coll* 1976).

Syngeneic bone marrow cells injected to supralethally irradiated mice will according to COVELLI *et coll* enhance the short term survival of the irradiated animals. On the other hand the life span of the survivors is reduced mainly on account of an increased incidence of degenerative and neoplastic diseases. It has been shown by KAPLAN & BROWN (1952), KAPLAN *et coll* (1953), WALLIS *et coll* (1966) and JÄRPLID (1968) that the incidence of lymphoreticular tumours (LR tumours) may be considerably reduced by shielding parts of the bone marrow during irradiation or by injection of bone marrow cells within a certain interval after whole body irradiation.

To which extent these modifications also are valid after contamination with nuclides do not seem to have been of much interest. It has been well documented (NILSSON 1962, 1970, NILSSON & BROOMÉ KARLSSON 1976, JÄRPLID 1973) that bone seeking internal emitters may have a very destructive effect upon the haematopoietic system and of immunologi-

cally potent cells as well (NILSSON 1970, JÄRPLID 1973, HALLER & WIGZELL 1977). Such animals will develop signs of a bone marrow aplasia such as general anaemia, haemorrhagic diathesis and malfunctions of the specific and unspecific defence system of the body as well as a high incidence of neoplasia mainly osteosarcomas and LR tumours.

The aim of the present report was to investigate if syngeneic bone marrow cells or thymic cells given to ^{90}Sr irradiated mice could influence upon factors such as life span, tumour incidence and tumour induction time of mainly osteosarcomas and LR tumours.

Materials and Methods

The animals were divided into two experimental series designed A and B containing 200 female and 250 male CBA mice respectively (Table 1). Carrier free ^{90}Sr (NO_3) was given to each of the series intraperitoneally at the age of 75 ± 5 days. The subdivision of the main series into groups (I-VIII) and their irradiation with ^{90}Sr and treatment with suspensions of bone marrow and thymic cells are recorded in Table 1. Since the bone marrow cavity is a very hostile environment for invading transplanted cells particularly during the first few months after ^{90}Sr injection two dose levels 25 m and 14.8 kBq (0.7 and 0.4 μCi)/g body weight were chosen in order to differentiate the radiation milieu for the homing

Table 1

Age of the mice at start of the experiment 75±5 days Day 0 Day of $^{90}\text{Sr}(\text{NO}_3)_2$ administration Intravenous dose of bone marrow (BM) cells 5×10^6 cells Intravenous dose of thymic (TH) cells 5×10^6 cells

Experiment	Group of mice	No of mice	Sex	Dose of $^{90}\text{Sr/g b w}$		Cell transplantation (days after ^{90}Sr inj.)								
						BM-cells			TH cells					
				μCi	kBq									
A	I	50	F	0.7	25.9	—								
	II	50	F	0.7	25.9	30	60	90	210					
	III	100	F	0.7	25.9	30	60	90	210	30	60	90	210	
B	IV	50	M	0.0		—								
	V	50	M	0.4	14.8	—								
	VI	50	M	0.4	14.8	1	30	60	90	Every month				
	VII	50	M	0.4	14.8	—								
	VIII	50	M	0.4	14.8	1	30	60	90	Every month	1	30	60	90

Every month The mice were given cell transplants monthly during their whole life span

Eighteen animals lost at the cell transfusion

Three animals lost

One animal lost

Five animals lost

Table 2

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of female mice (experiment A) treated with ^{90}Sr + bone marrow cells and ^{90}Sr + bone marrow cells + thymic cells. Dose of ^{90}Sr 25.9 kBq (0.7 μCi)/g b. Dose of bone marrow and thymic cells 5×10^6 . The cells were given every month between one and 7 months.

Group of mice	No of mice	Survival time (M±SE)	Dead without neoplasia	No of osteosarcoma bearing mice (per cent)	No of osteosarcoma bearing mouse	Survival time (M±SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Survival time (M±SE)
I (^{90}Sr)	50	770±9.0	0	47 (94.0)	4.45	276±8.8	209	3 (6)	253
II (^{90}Sr +BM)	50	254±8.8	0	39 (78.0)	4.00	277±7.8	156	16 (32)	184
III (^{90}Sr +BM+TH)	50	287±7.9	0	79 (96.3)	4.48	291±7.8	354	4 (4.9)	300

Five also had osteosarcoma

One also had osteosarcoma

Eighteen animals were lost at the cell transfusion

cells and their possibilities to colonize the bone marrow.

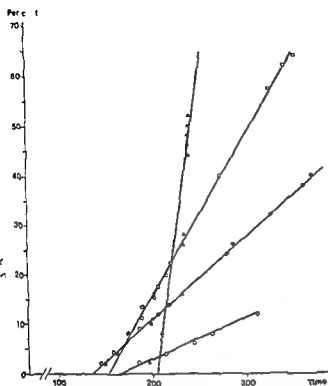
During the whole experiment the mice were kept in the same room in plastic cages 10 animals in each with free supply of food and water (Astra Ewos Standard Feed for Rats and Mice).

The bone marrow cells were harvested from syngeneic female CBA mice 75 to 100 days of age. After cutting both ends of 8 femurs the bone marrow was expelled by pressurized air and suspended in 0.5 ml of balanced salt solution (BSS). Then an additional 1.5 ml of BSS was added. From this suspension 0.1 ml was mixed with 50 ml BSS and 0.25

ml Zaponine and counted in a Coulter Counter. From each femur generally 5×10^6 cells could be obtained. From the stock suspension each mouse was given 0.25 ml intravenously adjusted so that the volume contained 5×10^6 bone marrow cells.

The thymus was cut in small pieces, homogenized in 0.5 ml of BSS and then handled in the same way as described for the bone marrow. Thymic cells were given intravenously in dose 5×10^6 cells suspended in 0.25 ml BSS.

All mice were if possible killed in agony and before autopsy examined roentgenologically in the dorso ventral position. The weight of the thymus



Cumulative incidence of lymphoreticular tumours in relation to time (days) after treatment with ^{90}Sr (●) ^{90}Sr + bone marrow cells (○) ^{90}Sr + thymus cells (□) ^{90}Sr + bone marrow + thymus cells (△)

and spleen as well as that of the whole carcass was recorded. Material was taken for histology from all cases with possible LR tumours and routinely both humeri tibiae femora pelvic bones the spine and the skull. The material was fixed in Steves solution embedded in paraplast cut at $5\text{ }\mu\text{m}$ and routinely stained with Hematoxylin Eosin and according to the van Gieson method.

Results

Experiment A The most obvious finding in series A (Table 2) was the high frequency of LR tumours in group II treated with bone marrow cells 16 of 50 animals (32%) whereas group III given both thymic and bone marrow cells had an incidence of only 4 of 82 (4.9%). The tendency to a prolonged latency time in the latter group is also notable. In group I given only ^{90}Sr the incidence of LR tumours was 6 per cent and the difference between group I and II is statistically significant ($\chi^2=46.53$ $p<0.0005$).

The number of osteosarcoma bearing mice and the total number of osteosarcomas are recorded in Table 2. From this it is evident that the tumour frequency was about 17 per cent lower in the bone marrow treated group (II) as compared to the other groups and further that the tumour frequency expressed as the number of osteosarcomas/osteosarcoma bearing mouse was about 10 per cent lower in group II (4.0) than in the other two groups (4.5). The survival time for the mice with osteosarcoma was practically the same in all groups in this series.

Experiment B In this series (Table 3) the LR tumours were more numerous by a factor 3.4 to 5 in all the groups (VI-VII-VIII) treated with cells in addition to ^{90}Sr as compared to the group given ^{90}Sr alone (V). The very high frequency of LR tumours 60 per cent found in the group treated with thymic and bone marrow cells was in a sharp contrast to the low value found in the group treated in a similar way in series A (4.9%).

Even if the induction time for the LR tumours is almost the same in all groups the induction pattern differs (Figure). As indicated here the first cases of

Table 3

Frequencies of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of male mice (experiment B): untreated treated with ^{90}Sr ^{90}Sr + bone marrow cells ^{90}Sr + thymic cells and ^{90}Sr + bone marrow cells + thymic cells. Dose of ^{90}Sr 14.8 kBq (0.4 $\mu\text{Ci/g}$ b.w.). Dose of bone marrow and thymic cells 5×10^6 cells. The cells were given every month with beginning at day 1 after ^{90}Sr injection.

Group of mice	No of mice	Survival time (M \pm SE)	Dead without neoplasia	Survival time (M \pm SE)	No of osteosarcoma bearing mice (per cent)	Survival time (M \pm SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Survival time (M \pm SE)
IV (Control)	47	810 \pm 15.7	45	—	—	—	—	2 (4)	531
V (^{90}Sr)	49	391 \pm 17.7	13	318 \pm 37.1	30 (61)	451 \pm 17.6	49	6 (12)	749 \pm 18.4
VI (^{90}Sr +BM)	49	335 \pm 19.7	17	798 \pm 27.8	12 (24)	512 \pm 20.3	20	20 (41)	260 \pm 15.7
VII (^{90}Sr +TH)	45	46 \pm 8.8	15	237 \pm 17.4	4 (4)	725	2	29 (64)	253 \pm 10.3
VIII (^{90}Sr +BM+TH)	50	257 \pm 11	15	246 \pm 7.7	5 (10)	409	8	30 (60)	231 \pm 2.4

LR tumours are observed at approximately the same day in both the groups given only one type of cells whereas in the series given both thymic and bone marrow cells the first neoplasia will appear about 50 days later. The slopes of the curves also indicate that the tumour progression rate is strongly increased in all groups given cells as compared to the group given ^{90}Sr alone. This is particularly obvious in the bone marrow + thymus cell group which exhibits a curve with a very steep slope and with a yield of 30 tumours within 65 days.

The majority of the LR tumours obtained were judged by histology of bone marrow origin. A primary observation was the early involvement of the bone marrow in the thoracic vertebrae with or without infiltration of the surrounding tissue. The soft tissue invasion was sometimes macroscopically detectable as strands bilaterally along the ventral aspect of the thoracic spine or later on as large greyish nodules. In the early phases the engagement of the spleen or other lymphoid tissue was insignificant. In more advanced stages however when the process was more widespread the spleen and lymph nodes could be enormously enlarged but still without macroscopic signs of thymic involvement. Of a total of 23 and 87 LR tumours in series A and B respectively only 2 definite thymic lymphomas were found in the former series and 4 in the latter.

As a consequence of the high rate of LR tumours the mean life span was significantly reduced for the animals in group VII ($t=9.139$ $p<0.001$) and group VIII ($t=6.635$ $p<0.001$) as compared to those given ^{90}Sr alone. The short life span in these groups also reflects the very low osteosarcoma incidence. In the group given ^{90}Sr + bone marrow cells (VI) with an intermediate not significantly shortened survival time the frequency of osteosarcomas is higher but still much less than in the group given ^{90}Sr alone; this also seems to depend largely on the high frequency of LR tumours. Taking into consideration only the number of osteosarcomas/osteosarcoma bearing mouse no difference exists between the two groups (V 1/63 and VI 1/66 tumours/mouse). However it should be observed that the tumour induction time is significantly longer for group VI 512 ± 20 3 days than group V 451 ± 17 6 days ($t=2.290$ $0.05 < p < 0.02$).

The results obtained from the registration of the body and organ weights of the dead animals did not reveal any significant differences between mice treated and not treated with cells. This was mainly

due to the large range of the weights of the individual mice and organs.

Discussion

The distribution of ^{90}Sr in the body has been subjected to a comprehensive whole body autoradiography investigation by NILSSON *et coll.* (1967). The nuclide is after a few hours selectively concentrated in the hard tissues. Initially it is mainly located in the metaphyseal parts and along the endosteal and periosteal linings of the bones. Later a redistribution takes part. After about 4 months the majority of the nuclide that remains in the mouse (approximately 20%) is now mainly retained in the diaphyseal regions whereas that retained in the metaphyseal zone has diminished considerably. This redistribution of the nuclide is the main reason why the bone marrow lesions vary in different regions of the bone at different times after its administration (NILSSON 1962, 1970).

After the doses of ^{90}Sr used the lymphohæmatopoietic tissues are severely damaged. This is evidenced by a marked dose related depletion of all cell types in the bone marrow and as a consequence a rapid involution of the thymus and a weight increase of the spleen due to a compensatory extramedullary hæmatopoiesis of long duration (NILSSON 1962, 1970, JÄRPLID 1973). In the bone marrow the cell depletion is most prominent after about one month when also a shift in the relative proportion between the granulocytic and erythrocytic series in favour of the former takes place whereas more and more of the erythroid cells and thrombocytes are produced in the spleen. In the bone marrow the cellular depletion has a sectional distribution which is most conspicuous in the metaphysis during the early phase after ^{90}Sr injection while later on the depletion predominates in diaphyseal areas. From day 120 to 150 a regeneration of all types of cells usually starts in the bone marrow however still with a predominance for the myeloid series. The bone marrow regeneration is most prominent in certain areas such as tubular, tubular, tubular, the thoracic vertebrae and in regions around the metaphysis. In late stages the diaphyseal parts of the bone marrow is hypoplastic and seldom regenerates at doses around the highest dose level used in the present experiments.

The decreased weight of the thymus is predom-

nantly caused by a cortical depletion of lymphoid cells characterized by a diphasic pattern with a deep initial depletion followed by a sharp abortive regeneration with its peak on day 12. Thereafter the cellularity decreases until about day 30 when the weight is normalized (JARPLID 1973). Also in the spleen a strong and persistent decrease of the lymphoid tissues occurs but on account of the heavy extramedullary haematopoiesis the spleen weight will be continuously elevated and for the doses used normalization of the weight occurs first after about 50 days (JARPLID 1973). This is mainly due to a more or less severe progressive lymphoid hypoplasia which may develop into secondary disease. According to JACOBSON (1954) the haematopoietic reserve offered by the spleen is one reason why the mice could survive the initial haematologic crisis since splenectomized mice will die.

In the present experiments a very high yield of LR tumours was obtained which is in complete contradiction to previous investigations with external irradiation which show that syngeneic bone marrow is very effective in protecting mice from the induction of lymphoma, myeloid leukaemia and reticular cell sarcoma (KAPLAN & BROWN, KAPLAN & COLLINS, COSGROVE & COLL 1964). This seems to a large extent to depend on the repeated supply of cells during the course of the experiment to mice which were continuously irradiated from ^{90}Sr located in the skeleton. The majority of mice were supplied with cell numbers from 25×10^6 to 100×10^6 . This is a fairly large number if it is considered that a crude approximation will give a total number of about 200×10^6 bone marrow cells in a normal mouse. Since the cells also were given from the very beginning of the experiment—in order to improve the general condition of the animals—most of the cells which were seeded within the bone marrow cavities were probably killed in the high dose series together with most of the bone marrow cells of the host (or severely damaged) during the first few months by the irradiation. This seems to be confirmed both by the lower incidence of LR tumours in experiment A (high dose group) as compared to B and by the fact that the latency time for LR tumours (in series B) is about the same in cell treated and not cell treated ^{90}Sr groups. First later when the concentration of ^{90}Sr was diminished certain sites of the bone marrow would offer an environment allowing regeneration and then first in the groups given the lower ^{90}Sr dose. This means also that mice given cells will offer

a largely increased number of cells at risk as compared to those given ^{90}Sr alone which seems to be the main reason for the high incidence of LR tumours.

In these experiments the injected cells do not seem to colonize the spleen to the same degree as is the case after external irradiation (TILL & MCCULLOCH 1961). Virtually no spleen was found to contain colony forming units. This may be associated with the fact that the radiation damage to the spleen after ^{90}Sr treatment is only minor as compared to that after high external irradiation doses leaving the spleen with an almost intact capacity of regeneration and as a consequence an insignificant need of cell import. Therefore the potentially larger haematopoietic reserve of the spleen will soon be activated to overcome the acute bone marrow failure induced by ^{90}Sr . A reasonable anticipation is thus that the majority of the transplanted cells will migrate to the bone marrow which also may be supported by the fact that most cases of LR tumours seem to have a bone marrow origin. This is also in agreement with previous findings (NILSSON 1971) that the majority of ^{90}Sr induced LR tumours originate in the bone marrow.

The large difference in the incidence of LR tumours between group II and III in experiment A could not readily be explained but may be related to the site in the body where the thymic cells are seeded. Thymic cells are when injected intravenously distributed to all lymphoid organs and to the bone marrow (ZATZ & LANCE 1970). However these cells are extremely sensitive to irradiation and it would therefore be anticipated that the fraction of those homing in the bone marrow to a large extent will be exterminated and therefore not possibly constitute a large pool of potentially transformable cells. This is particularly valid in the highest—less tumorigenic—dose series with the lowest incidence of LR tumours. The thymic cells which are seeded in the spleen and lymph nodes or incorporated in the recirculating lymphocyte pool may survive for a considerable time (SPRENT & BASTEN 1973) thereby helping to reactivate the cell mediated immunologic defence and counteract transforming cellular clones.

The most efficient ^{90}Sr dose for induction of LR tumours seems to be somewhere between 14.8 and 7.4 kBq (0.4–0.2 μCi)/g of body weight (NILSSON 1971) and it is also known that female CBA mice are more susceptible than are males (JARPLID 1974). The somewhat lower incidence in group II series A as

Table 4

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of male mice (experiment C) treated with ^{90}Sr and ^{90}Sr + bone marrow cells. Dose of ^{90}Sr 25.9 kBq (0.7 μCi)/g b.w. Dose of bone marrow cells 5×10^6 cells

Group of mice	No. of mice	Survival time (M \pm SE)	Dead without neoplasia	Survival time (M \pm SE)	No. of osteosarcoma bearing mice (per cent)	Survival time (M \pm SE)	Total No. of osteosarcomas	No. of lymphoreticular tumours (per cent)	Survival time (M \pm SE)
I (^{90}Sr)	98	314 \pm 6.8	7	246 \pm 24.8	85 (86.7)	378 \pm 6.1	194	8 (8)	0 (0)
2 (^{90}Sr + BM ₁₂₀)	98	312 \pm 7.0	19	248 \pm 17.1	74 (75.5)	336 \pm 5.4	166	8 (8)	76 (77)
3 (^{90}Sr + BM ₁₇₀)	97	339 \pm 10.4	15	361 \pm 59.3	75 (81.5)	336 \pm 5.5	190	5 (5)	6 (6)

BM₁₂₀/BM₁₇₀ indicates the day bone marrow cells were given after the day of ^{90}Sr administration

Three also had osteosarcoma

Two also had osteosarcoma

Two animals lost

Eight animals killed when cells were injected

compared with group VI series B may be related to these facts i.e. a considerably higher frequency of LR tumours would have been expected in group A II if the dose had been 14.8 kBq (0.4 μCi)/g. The highest incidence of LR tumours (64%) is observed in series B group VII which was given thymic cells alone. The group given both thymic and bone marrow cells in series B had in contrast to the corresponding group in series A a high incidence of LR tumours (60%). As already mentioned 14.8 kBq (0.4 μCi)/g is a more tumorigenic dose regarding LR tumours than 25.9 kBq (0.7 μCi)/g largely because of lower cell killing effect. In the lower dose group a larger number of potentially transformable cells therefore will survive making the cellular defence even if enhanced insufficient to counteract neoplastic transformation.

LR tumours usually originate in the bone marrow when ^{90}Sr is given within optimum dose limits (NILSSON 1971, JARPLID 1974). This observation is in agreement with the findings in the present investigation and indicates that the cell transplantation could not modify the tumorigenic mechanism in this respect. In this context it seems necessary to implicate that the increased incidence of LR tumours may be associated with a virus activation by the injected cells. However no attempts have been made to unravel this possibility.

On account of the high frequency of LR tumours in the cell treated groups in series II most animals died before osteosarcomas could develop. However this was only partly the case in group VI where osteosarcomas developed in 12 mice as com-

pared to 30 in group V. In both these groups number of osteosarcomas/osteosarcoma bearing mouse was the same 1/6. The significantly longer survival time of the bone tumour bearing mice group VI may perhaps be considered as an indication however vague of a reinforcement of the host antitumour defence. The findings in series A that number of bone tumours per mouse was slightly higher in group II than in group I (Table 2) may also be taken as a weak indication in this direction supported by the results in group III however. Since these observations were difficult to evaluate and partly were contradictory a new experiment series C—was inserted in which modifications were undertaken as regards the most convenient time for bone marrow transplantation and number of cells injected. Three groups each of 100 CBA male mice were given 25.9 kBq (0.7 μCi) ^{90}Sr /g b.w. intraperitoneally. One group (1) was given only ^{90}Sr and the other two were given 5×10^6 bone marrow cells in a single intravenous injection on day 120 (2) and 170 (3) respectively after the nuclide administration. The results of these experiments are recorded in Table 5. The time 120 days was chosen because it seems to offer one of the earliest opportunities for the transplanted cells to successfully repopulate the bone marrow. Day 170 was selected also with the purpose to aim to interfere with the earliest preneoplastic proliferations which with the doses administered do occur at this time (NILSSON 1970) with the exception of the rare occasions of earlier appearance.

In the inserted experiment C a few observations were made which may help to explain the results.

the original investigation. Thus the frequency of LR tumours was not enhanced in the cell treated groups because in contrast to the earlier experiments precautions were taken not to provoke tumorigenesis by giving the transfusion as a single dose long enough after ^{90}Sr to assure a less risky environment to the transplanted cells. Consequently the number of cells at risk was held within limits. A slight indication of a beneficial effect of cell treatment may also appear from an increased life span for the animals given cells at day 170 (versus ^{90}Sr only $t=2.0113$ $0.02 < p < 0.05$). Also animals dying without neoplasia show a tendency to have a longer life span in group 3 than in group 1 (however not significant $t=1.788$ $p=0.10$).

From the results of experiment A and B the anticipations arose that cell injections may have a stimulatory effect on the antitumour defence. This conclusion is very uncertain and could not be unambiguously answered by the inserted experiment nor is it supported by COSGROVE *et coll.* or COVELL *et coll.* who reported that neoplasms other than LR tumours were practically unaffected by bone marrow transplantation. A χ^2 analysis of the tumour material in Table 4 reveals an almost significantly lower tumour frequency if group 1 is compared with group 2 ($\chi^2=5.272$ $0.010 < p < 0.025$) whereas group 1 versus 3 does not fulfil the statistical standards of significance ($\chi^2=2.712$ $p=0.10$).

Whatever the truth may be as regards the possibility to reactivate the haematopoiesis, the specific and unspecific defence system as well as the general condition of heavily contaminated animals, it seems necessary to point out the enhancing risk of LR neoplasia when transplanting lympho-haematopoietic cells to individuals contaminated with bone seeking nuclides.

It also seems to be of a biologic interest to investigate more exactly the optimum injection time and cell doses which can be given without exacerbating the induction of LR tumours but utilize the beneficial effect which the present experiments indicate. In this context it also seems to be of interest to analyse if and in which way bone marrow transplants may increase the population of the very ^{90}Sr sensitive natural killer cells (HIESLING & WIGZELL 1979) in animals irradiated with this nuclide at different times after ^{90}Sr injections and to what an extent these cells during more optimum conditions may be able to counteract tumour progression.

SUMMARY

Mice irradiated with ^{90}Sr in doses of 14.8 and 25.9 kBq/g body weight were given bone marrow or thymic cells intravenously at monthly intervals or in single doses at 120 or 170 days after injection of ^{90}Sr . At the low dose level a high incidence of lymphoreticular tumours in all cell treated groups occurred as compared with animals irradiated with ^{90}Sr only. At the high dose level only the bone marrow transplanted group contracted a high incidence of lymphoreticular neoplasia. Furthermore a somewhat decreased osteosarcoma incidence in the cell transplanted animals appeared to be indicated. However the results obtained are inconsistent and difficult to evaluate. Therefore it seems necessary to repeat the experiments if more precise conclusions should be possible to draw.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Swedish Cancer Society (Project No. 790-B77 03XC) for the support of this report. The investigation was also carried out as part of the program of the European Late Effects Project Group (EULEP).

REFERENCES

- BARNES D W H, BUNYAY G T and MOLE R H. Delayed mortality of midlethal exposures to whole body irradiation and its modification by treatment with syngeneic lymph node or bone marrow cells. *Int J Radiat Biol* 11 (1966) 409.
- COLE L J, OBERMAEYER J G and BOND V P. Recovery from acute radiation injury in mice following administration of rat bone marrow. *J Nat Cancer Inst* 16 (1955) 1.
- CONGDON C C, MAKINODAN T and GENOZOZIAN N. Effect of injection of rat bone marrow on reticular tissues of mice exposed to X radiation in the midlethal dose range. *J Nat Cancer Inst* 18 (1957) 603.
- COSGROVE G E, UPTON A C, CONGDON C C, DOHERTY D G, CHRISTENBERRY K W and GOSSLEE D G. Late somatic effects of γ radiation in mice treated with AET and isologous bone marrow. *Radiat Res* 21 (1964) 550.
- COVELL V, METALLI P, BRIGANTI G, BASSINI B and SILINI G. Late somatic effects in syngeneic radiation chimaeras. II. Mortality and rate of specific diseases. *Int J Radiat Biol* 26 (1974) 1.
- DUPLAN J F, RECHTNIKOV V P and KOSINETZ G. Effet de la moelle osseuse homologue provenant de donneurs tolérante sur la survie de souris irradiées. *C R Soc Biol* 156 (1962) 1773.
- HALLER O and WIGZELL H. Suppression of natural killer cell activity with radioactive strontium. Effector cells are marrow-dependent. *J Immunol* 118 (1977) 1503.
- JACOBSON L O. The haematological effects of ionizing radiation. In *Biology Vol 1* Edited by A. Hollaender. McGraw Hill, New York, Toronto, London 1954.

Table 4

Frequency of macro- and microscopic osteosarcomas and lymphoreticular tumours in groups of male mice (experiment C) treated with ^{90}Sr and ^{90}Sr + bone marrow cells. Dose of ^{90}Sr 25.9 kBq (0.7 μCi)/g b.m. = Dose of bone marrow cells 5×10^6 /cells

Group of mice	No of mice	Survival time (M \pm SE)	Dead without neoplasia	Survival time (M \pm SE)	No of osteosarcoma bearing mice (per cent)	Survival time (M \pm SE)	Total No of osteosarcomas	No of lymphoreticular tumours (per cent)	Survival time (M \pm SE)
1 (^{90}Sr)	98 *	314 \pm 6.8	7	246 \pm 24.8	85 (86.7)	378 \pm 6.1	194	8 (8)	0 (0)
2 (^{90}Sr + BM ₁₂₀)	98 *	312 \pm 7.0	19	248 \pm 17.1	74 (75.5)	336 \pm 5.4	166	8 (8)	236 \pm 7.1
3 (^{90}Sr + BM ₁₇₀)	92	339 \pm 10.4	15	361 \pm 59.3	75 (81.5)	336 \pm 5.5	190	5 (5)	67 \pm 3.1

BM₁₂₀/BM₁₇₀ indicates the day bone marrow cells were given after the day of ^{90}Sr administration

Three also had osteosarcoma

Two also had osteosarcoma

Two animals lost

Eight animals killed when cells were injected

compared with group VI series B may be related to these facts. In a considerably higher frequency of LR tumours would have been expected in group A II if the dose had been 14.8 kBq (0.4 μCi)/g. The highest incidence of LR tumours (64%) is observed in series B group VII which was given thymic cells alone. The group given both thymic and bone marrow cells in series II had in contrast to the corresponding group in series A a high incidence of LR tumours (60%). As already mentioned 14.8 kBq (0.4 μCi)/g is a more tumorigenic dose regarding LR tumours than 25.9 kBq (0.7 μCi)/g largely because of lower cell killing effect. In the lower dose group a larger number of potentially transformable cells therefore will survive making the cellular defence even if enhanced insufficient to counteract neoplastic transformation.

LR tumours usually originate in the bone marrow when ^{90}Sr is given within optimum dose limits (NILSSON 1971, JARPLID 1974). This observation is in agreement with the findings in the present investigation and indicates that the cell transplantation could not modify the tumorigenic mechanism in this respect. In this context it seems necessary to implicate that the increased incidence of LR tumours may be associated with a virus activation by the injected cells. However no attempts have been made to unravel this possibility.

On account of the high frequency of LR tumours in the cell treated groups in series B most animals died before osteosarcomas could develop. However this was only partly the case in group VI where osteosarcomas developed in 12 mice as com-

pared to 30 in group V. In both these groups the number of osteosarcomas/osteosarcoma bearing mouse was the same 1.6. The significantly longer survival time of the bone tumour bearing mice in group VI may perhaps be considered as an implication however vague of a reinforcement of the host antitumour defence. The findings in series A that the number of bone tumours per mouse was slightly less in group II than in group I (Table 2) may also be taken as a weak indication in this direction not supported by the results in group III however. Since these observations were difficult to evaluate and partly were contradictory a new experiment—series C—was inserted in which modifications were undertaken as regards the most convenient time for bone marrow transplantation and number of cells injected. Three groups each of 100 CBA male mice were given 25.9 kBq (0.7 μCi) ^{90}Sr /g b.m. intraperitoneally. One group (1) was given only ^{90}Sr and the other two were given 5×10^6 bone marrow cells in a single intravenous injection on day 120 (2) and 170 (3) respectively after the nuclide administration. The results of these experiments are recorded in Table 4. The time 120 days was chosen because it seems to offer one of the earliest opportunities for the transplanted cells to successfully repopulate the bone marrow. Day 170 was selected also with the purpose to aim to interfere with the earliest preneoplastic proliferations which with the doses administered do occur at this time (NILSSON 1970) with the exception of the rare occasions of earlier appearance.

In the inserted experiment C a few observations were made which may help to explain the results.

SIMPLE EXPERIMENTALLY DERIVED ALGORITHM FOR COMPUTER
CALCULATED DOSE RATES ASSOCIATED WITH
¹³⁷Cs GYNECOLOGIC INSERTIONS

D. E. WREDE and H. DAWALIBI

POWERS et coll compared the MIR (Mallinckrodt Institute of Radiology) program with three well known programs in 1966. The three programs were by SHALEK & STOVALL (1962-1968), ADAMS et coll (1964) and ADAMS & MEURK (1964). Radium was used as the nuclide. The second and third programs are for intracavitary treatment and the first program for interstitial treatment only.

Their programs were prompted by the fact that a difference in dose of the order of 20 per cent at a particular point occurred. An interesting point was illustrated, i.e. the distance to be moved for normalization of the dose for the several programs is only a few millimeters. Thus, because of the steep gradients that occur in intracavitary (and even more in interstitial) implants, large per cent differences may occur for either a calculated or measured dose rate at a specified point. This led to the conclusion that accuracy is best expressed in terms of distances to be moved in order to obtain agreement between different programs rather than trying to compare measured or calculated dose rates.

Another interesting observation they made was that the maximum at distances from a source equal to or greater than two times the length of the source, the dose variation is nearly approximated by an inverse square function, is nearly true and can be used as a rapid approximation of dose without the necessity of performing explicit calculations in the estimation of doses from intracavitary apertures.

Finally, they suggested that a rapid program would have a simple rapid and accurate input, a concise and accurate computational method using

reasonable factors and logic and a clearly meaningful output. This latter statement summarizes the basis for this report.

Conventional computational methods

One of the well known methods for intracavitary dose calculations is the SHALEK & STOVALL (1968) interval method for determining dose rate to a point P from a linear ¹³⁷Cs source and is based upon dividing the active length into N point sources and summing the contributions from each. The basic formula for one source is

$$(D_p)_i = \frac{\Gamma Cs A f}{N} \sum_{i=1}^N \left\{ \frac{e^{-\mu d_i}}{d_i} \right\} \times T(d_i) \quad (1)$$

where

$(D_p)_i$ = the dose rate at the point p from the jth source

ΓCs = the exposure rate constant for ¹³⁷Cs

f = rad/roentgen conversion factor for muscle tissue

A = the activity of the source

μ = the linear attenuation coefficient of the wall materials for ¹³⁷Cs photons

d = the distance from the point P to the center of the ith point source of the source

t_i = the path length within the filter for a ray from the ith point source

$T(d_i)$ = tissue correction factor compensating for absorption and scatter

N = number of point sources assumed per actual active length of a single source

This formula would be applied to all sources and the results all summed for point P

BREITMAN (1974) goes into some detail expanding upon the definition and empirical fitting of the terms mentioned and derives by computer calculation a table of dose rates for a variety of ^{137}Cs tube geometries. Active lengths are for 1.35 cm, 1.5 cm, 3.0 cm and 4.5 cm and for a variety of filtrations and active diameters as well.

Using tables published by SHALEK & STOVALL (1968) as a basis for comparison between radium sources and cesium sources having the same construction and equivalent radium content, the dose distributions were found to be similar with the exception of the heavily filtered ends where 9 per cent difference occurred due in part to the complex spectrum of radium.

When BREITMAN's result is compared with KRISHNASWAMY's theoretical data (1972) for stainless steel encapsulation, agreement is found only on the transverse axis. KRISHNASWAMY's tables were derived from MIRD (Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine) data according to BERGER (1968). The two methods agreed to within one per cent at most points when the computer method was used to generate a table for a steel filtered cesium needle using a linear energy absorption coefficient of 0.221 cm^{-1} based on HUBBELL's data (1960). This procedure confirmed (1) the equivalence of the two approaches to calculating dose rates and the conclusion that the dose distribution of platinum filtered ^{137}Cs sources is more closely related to that of platinum filtered ^{226}Ra sources of the same size than to stainless steel filtered ^{137}Cs sources. Actually the first part of this conclusion was determined much earlier by HORTWITZ et al. (1964) and others including the first author. An explicit simple power expression for dose rate was derived from a ^{137}Cs point source in an infinite unit density medium based upon the Monte Carlo derived data (BERGER) after observing that a plot of log of specific absorbed fraction versus the log of distance from the point source formed a straight line.

A least squares fit gave the expression

$$D = \frac{k_1}{d^k} \times A \quad (1)$$

where D = dose rate (Gy/h) in an infinite unit density medium at a point d cm from the point source of ^{137}Cs of A activity (in Bq) and k_1 and k are constants obtained from a least squares fit.

Computer algorithm

Using the theoretically derived expression for dose rate from a point source, a simple program can be written in which a simple do loop is utilized to break the source up into any number of pieces such that each piece i of linear source j can be treated as a point source. The total dose rate to a given point of interest p is thus given by a simple numerical integration i.e.

$$D_p = \sum_{j=1}^N \sum_{i=1}^M \frac{k_1 A_i}{d_{ij}^k} \quad (2)$$

where

$$d_{ij} = [(X_{ij} - X_p)^2 + (Y_{ij} - Y_p)^2 + (Z_{ij} - Z_p)^2]^{1/2} \quad (3)$$

X_{ij} , Y_{ij} , Z_{ij} = the coordinates of the i th point source of the j th source

X_p , Y_p , Z_p = the coordinates of the point of interest

A_{ij} = the activity of the i th point source of the j th source

M = the number of linear sources

N = the number of point sources specified per each real source active length

D_p = the total dose rate at point p from all sources

Program features such as the provision to go back and easily correct coordinates that were typed incorrectly into the terminal, the ability to modify source strengths and thus optimize point A-rectal (or bladder) dose rate differentials, use of the conversational mode, the ability to print out dose rates in specified planes of interest (one centimeter actual spacing), etc. provide a useful versatile program. Basically the only input data required are: (1) The x , y , z coordinates at the end of each active length, (2) the activity in mCi of each source, (3) the lateral and a p radiographic magnification factor, (4) the source decay factor, and (5) spatial coordinates of points of

interest Data can be extracted from a set of orthogonal films inputted into a conventional teletype terminal and outputted all in less than half an hour. The same program can of course also be implemented on a small dedicated type computer and the coordinates inputted using a digitizer.

Comparison between theoretical dose rates

A comparison of theoretical dose rates for a wide selection of clinical points of interest between the present data and those of KRISHNASWAMY agreed within one per cent on the perpendicular bisector for a single 2 cm source of active length 12 cm. This is of no great surprise since both were derived from the same basic MIRD data according to BERGER. Slight differences of the order of 3 per cent occurred at points not lying on the transverse axis due to the fact that the present model did not include the effects of oblique filtration although it did inherently contain the effect of 0.5 mm of platinum on the transverse axis. In an attempt to retain the same simple analytical algorithm, experimental data were obtained from a Rando Alderson phantom and the constants k_1 and k_2 were adjusted in order to force agreement between the computer printout and the experimental data.

General experimental procedure

A ^{137}Cs source (OL=2.0 cm AL=1.2 cm filtration=0.5 mm platinum) was placed at approximately the external os position in the Rando Alderson phantom. LiF (TLD 700 powder) detectors were placed in the same place of the phantom i.e. on the perpendicular bisector of the source and in places above and below the source. Each slab was 2.5 cm thick but powder only occupied the central 1.2 cm portion of each slab corresponding to the active length of the source. Irradiations were carried out over both a 17 hour and an 18.5 hour time interval and averages of readings were recorded. The powder was read out on a Harshaw TLD Unit and readings were used to determine doses and therefore dose rates using a calibration curve based upon the known outputs of a ^{60}Co teletherapy unit. Readings were plotted against length of the powder and the peak at the center point was taken as the correct reading for determining the dose. A contrast medium (Hypaque) was next placed into the teflon capsules which had been used to hold the LiF powder

and the capsules repositioned into the phantom at the same positions which the LiF capsules had occupied. Anterior and lateral films were exposed and coordinates corresponding to the ends of the active source and centers of the Hypaque filled capsules were carefully measured from the film. (Actually all coordinates were known by geometry but the radiographic procedure allowed for a better simulation corresponding to the routine clinical practice.) The existing computer program was used to calculate dose rates at the measured points and these dose rates were compared with the directly measured dose rates.

Comparison of theoretical and experimental dose rates

Differences varied from 4 to 10 per cent between the experimental and computer derived data. By adjusting k_1 and k_2 differences were minimized to within 2 per cent except at extreme oblique positions. The differences within a solid angle produced by $\pm 45^\circ$ relative to the transverse axis at the center of the source were less than 2 per cent. This is the same magnitude of difference when calculating with precisely known spatial coordinates as compared with coordinates obtained from a set of orthogonal films.

After adjusting k_1 and k_2 differences of dose rate along the transverse axis for a single source varied systematically from the data of KRISHNASWAMY the difference being 10 per cent at a distance of 7 cm. At a distance of 2 cm the difference was 5 per cent. In all cases the experimental (or computer generated) value after adjustment of k_1 and k_2 dose rates were always lower. This is intuitively expected since the theoretical model is based on an infinite medium and the experimental data are based upon a finite medium (the Rando Alderson phantom). As one proceeds further from the source less scatter component would be expected in the finite medium thus reducing the dose rate from that which would occur in an infinite medium.

Conclusion

POWERS et al. have suggested that an ideal program would have a simple, rapid and accurate input and a concise and accurate computation method using reasonable factors and logic as well as a clearly meaningful output. The authors feel as if the

present algorithm provides the basis for such a program. An analytical expression in lieu of table lookup allows conciseness, reduction of required computer memory and the accuracy of the computation is assured by the agreement ($\pm 2\%$) with experimentally derived data using a female Rando Alderson phantom. The input parameters are simple and can be quickly ascertained. Much of the accuracy depends on the individual spatial coordinates which can be obtained directly from a set of orthogonal films by hand or can be inputted directly using a transducer. The output is meaningful and useful in afterloading techniques if one allows for the printout of dose rates from individual sources as well as the summed dose rates from all sources for any point of interest. This is useful since the source strengths can easily be modified within the computer until more desirable differential dose rates are obtained. The results are also interesting in that not only is energy build up and attenuation accounted for in the unit density medium but finiteness of the medium appears to have an effect, reducing the dose rate with distance from the source as compared with theoretically expected values. In practice this is of no great consequence though since points of clinical interest such as points A, B, C, rectum, bladder etc. lie relatively close to the sources.

SUMMARY

A simple mathematical algorithm is derived from experimental data for dose rates from ^{137}Cs sources in a finite tissue equivalent medium corresponding to the female pelvis. An analytical expression for a point source of ^{137}Cs along with a simple numerical integration routine allows for rapid as well as accurate dose rate calculations at points of interest for gynecologic insertions. When compared with theoretical models assuming an infinite unit density medium, the measured dose rates are found to be systematically lower at distances away from a single source: 5 per cent at 2 cm and 10 per cent at 7 cm along the transverse axis. Allowance in the program for printout of dose rates from individual sources to a given point and the feature of source strength modification allows for optimization in terms of increasing the difference in dose rate between reference treatment points and sensitive structures such as the bladder, rectum and colon.

Request for reprints: Dr Don E. Wrede, Medical Physics Department, King Fahad Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh, Saudi Arabia.

Copies of the computer program written in Fortran are available from the authors upon request.

REFERENCES

- ADAMS G. D. and MEURA M. L. Use of computer to calculate isodose information surrounding distributed gynaecological radium sources. *Phys. in Med. Biol.* (1964) 533.
- ADAMS R. M., PETERSON M. D. and COLLINS V. P. Clinical useful calculations of dose distributions from multiple radiation sources. Presented at the Annual Meeting of the Radiological Society of North America, 1964.
- BERGER M. J. Energy depositions in water by photons from point isotropic sources. *MIRD* (1968) Supplement No. 1.
- BREITMAN K. E. Dose rate tables for clinical Cs^{137} sources sheathed in platinum. *Brit. J. Radiol.* 41 (1968) 657.
- HORWITZ H., KEREIAKES J. C., BAHR G. K., CLIVE S. E. and BARRATT C. M. An after loading system utilizing cesium 137 for the treatment of carcinoma of the cervix. *Amer. J. Roentgenol.* 91 (1964) 176.
- HUBBELL J. H. Photon cross sections, attenuation coefficients and energy absorption coefficients from 10 keV to 100 GeV. National Standard Reference Data Series 29. National Bureau of Standards, Washington D.C. 1960.
- ICRU Report 23. Measurement of absorbed dose in a phantom irradiated by a single beam of X or gamma rays. 1973.
- JOHNS H. E. and CUNNINGHAM J. R. The physics of radiology. Third Edition. Charles C. Thomas Springfield, Illinois 1969.
- KRISHNASWAMY V. Dose distributions about Cs^{137} sources in tissue. *Radiology* 105 (1972) 181.
- POWERS E. W., SCHNIEDER A. K., SHIVARATHA K., FOTY H. and GALLAGHER T. Evaluation of methods of computer estimation of interstitial and intracavitary dosimetry. *Amer. J. Roentgenol.* 96 (1966) 59.
- SHALFAR J. and STOVALL M. A. Calculation of dose for interstitial implantations. In *Radiation therapy in the management of cancers of the oral cavity and oropharynx*, p. 293. Edited by G. Fletcher and W. MacComb. Charles C. Thomas, Springfield, Illinois 1962.
- M. H. Anderson method for computation of isodose curves around interstitial and intracavitary radium sources. I. Dose from linear sources. *Amer. J. Roentgenol.* 102 (1968) 667.
- STOVALL M. A. and SHALFAR J. R. Study of the distribution of radiation in interstitial implantations. Method of calculation with automatic digital computer. *Radiology* 78 (1962) 950.

COMPUTATION OF DOSE DISTRIBUTIONS
FOR RADIOACTIVE SEED IMPLANTS

I I ROSEN II G LANE and C A KELSEY

Interstitial and intracavitary implants with radioactive seeds permit irradiation of localized tumors with higher doses to tumor and lower doses to surrounding normal tissue than are possible with external beams. Temporary implants are typically used for carcinoma of the cervix, vagina, rectum, oral cavity, and oropharynx. Permanent implants are typically used for malignancies in such sites as the prostate, lung, bladder, and lymph nodes. Some review articles on implantation technique, dosimetry, and clinical results include ANDERSON (1975), SYED & FEDER (1977), HILARIS (1968, 1976), HILARIS et al. (1974, 1975, 1976), and LIM & HILARIS (1975).

Tumor and normal tissue doses from an implant are sensitive to source geometry because of the localized distribution of dose from each seed. Although some of these implants can be approximated by models, each implant is unique and in general requires a dose calculation based on the actual source configuration. In principle, dose distributions of an implant could be calculated by hand from the distribution of the individual seeds; however, in practice, hand calculations for doses at more than a few points are impractical. STOVALL & SHALEK (1972) have summarized the computer methods used for all types of interstitial and intracavitary dosimetry.

Material and Methods

An interactive computer program for computation of dose distributions from radioactive seed implants

is described. The program can handle up to 300 ^{125}I or ^{192}Ir seeds. Seed coordinates can be entered using either the keyboard or a sonic digitizer from either orthogonal or stereo films. The program produces a data file containing a dose matrix. This output data file can be combined with the output of other treatment planning programs to provide total treatment plans for combined modalities. Separate programs display and plot the isodose distributions. The last case run is saved in a temporary file; therefore, until a new case is entered, the last case can be recalled easily for additional plans or for modification and new plans if the implant was changed. The program is run on a PDP 11/40 computer from a Tektronix graphics terminal. A Versatec printer/plotter is used for hardcopy alphanumeric output, and the isodose distributions are plotted on a Houston Instruments pen plotter.

The program manipulates seed information in units of ribbons, although this term is not applicable to ^{125}I sources. Each ribbon is defined by the user and may have from 1 to 300 seeds; therefore, a ribbon may consist of a single seed, an actual ribbon (^{192}Ir), an entire plane, or even the entire implant. All seeds in a given ribbon have the same activity; however, the activity of the seeds in different ribbons may be different. The source coordinates are entered in groups by ribbon.

When the sonic digitizer is used for computer input, the origins and axes of the films need be entered only once. They can be re-entered if the films have

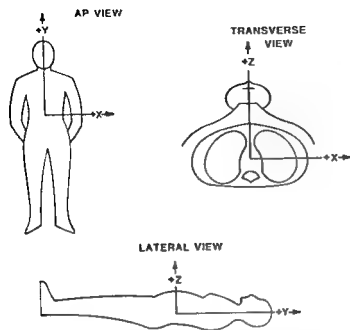


Fig 1 Reference coordinate system. The standard planes of calculation. Arbitrary planes of calculation are specified by the coordinates of three points relative to this coordinate frame.

been moved between digitizing ribbons. The number of seeds in a ribbon is entered at the keyboard, and the number entered via the digitizer is checked against the number expected.

For ^{192}Ir seeds, the activity of each seed is entered in equivalent mg of radium. The program then computes a dose rate distribution in Gy/h. The dose rate at 1 cm in tissue is calculated from the gamma factor and f factor to be 0.0792 Gy/mg h (7.92 rad). Doses at all other points are calculated using the inverse square law.

The activity of ^{131}I seeds is entered in MBq. The dose rate as a function of distance in Gy/(mCi)/h is tabulated from the graph given by ANDERSON. The dose rates at all points are computed from the table using linear interpolation for intermediate values. Then because these implants are permanent the dose rate at each point is multiplied by the mean life of ^{131}I (2073.6 h) to give the dose at that point resulting from the total decay of the seeds.

A reference coordinate system for all seed positions is used based on standard antero-posterior, lateral and transverse views (Fig 1). These standard isodose plans, including displacements from the origin, may be requested directly. Arbitrary planes of calculation are specified by three points

Seed

Te 1 case 1		00-00-00
Ir	0.10 to 0.30 mg	
21 seeds in	3 ribbons	
Seed coordinates	from stereo films	
Target film-d stance	= 120 cm	
Displacement angle	= 8 degrees	
1 isodose plan		
EN	Enter a new case (change all)	
SA	Save this case permanently	
CF	Change film factors	
DR	Delete a ribbon—type number	
AR	Add a ribbon	
CA	Change activity of a ribbon—type number	
LS	List the seeds—type ribbon number (0 all)	
PS	Print the seeds on the Versatec	
CS	Change a seed—type number	
LP	List the isodose plans	
DP	Delete a plan—type number	
AP	Add a plan	
CO	Continue with the calculation	
—	End the program	
Option		

Fig 2 Main display of the program. A summary of the data entered with a list of command options. Some of the options give detailed information about the implant.

whose coordinates are given using the patient coordinate frame. The location of the coordinate system origin is arbitrary. However, the y axis and the position of the origin must be the same on both films of an orthogonal or stereo pair.

The implant films are taken on a Picker isocenter simulator. For orthogonal films, the isocenter is placed at the center of the implant. Antero-posterior and lateral films are exposed sequentially by rotating the unit. The magnifications are computed from the source-to-axis and source-to-film distances. For obtaining stereo films, the patient is placed on a support frame with a space in it for the film cassette. The film is located on the table top directly below the patient. The patient is raised so that the isocenter of the machine is set at the film. The center of the field is therefore the same point in space for both stereo films and is used as the coordinate origin when digitizing the films. Since the isocenter appears on the films, no additional marking is needed. The same displacement angle from the vertical about the y axis is used for both stereo films. Typically, a source-to-film distance of 120 cm and a displacement angle of 8° are used.

Standard antero-posterior, lateral and transverse isodose plans can be requested by a single command.

of the plan may be entered or the computer calculates the average value of all the actual seed rates. Displacements from the origin can be entered to give sectional views. The grid limits the area over which the doses are to be calculated. They may be entered or the computer will calculate grid limits that are at least 2 cm greater than the most extreme source coordinates in each direction, rounded off to the nearest centimeter.

For both orthogonal and stereo films the y-coordinate of a source is independently computed from the film. An uncertainty in the value Δy is then calculated. This uncertainty is displayed or printed in addition to the source coordinates. This delta Δy is useful in determining if the patient moved between the films or if a digitizing error occurred. The y value used for computing isodose distributions is the average of the two independent y values.

The main display of the program appears in Fig. 2. Letter mnemonics are used for specifying command options. Where a seed ribbon or plan number is required that number may be entered with the letter and mnemonic. For example, isodose plan number 1 can be deleted by typing DPl. If the number is omitted the computer will issue an appropriate prompt. The available commands are listed upon as follows:

Enter all new information. If an error is encountered in retrieving the last case, this option is automatically invoked.

Save this case in a permanent file. The permanent file, intended for statistical or other retrospective analysis, will be named according to the patient's name and the isotope used.

Change the film factors. The factors changed depend on whether the films are orthogonal or stereo. This option will cause a recomputation of the actual coordinates and the Δy values of the sources.

Delete a ribbon.

Add a ribbon.

Change the activity of a ribbon.

List the seed data on the terminal. The data are listed by ribbon with the ribbon activity shown. Such data include the film coordinates, actual coordinates, and Δy for each seed.

Print the seed data on the printer. The same information as that in the LS command is printed.

- CS Change the coordinates of a single seed.
- LP List the isodose plans to be calculated on the terminal. The information presented is the plan description, the coordinates of the three points defining the plane, and the calculation grid limits.
- DP Delete an isodose plan.
- AP Add an isodose plan. Up to seven isodose plans can be specified. Standard antero-posterior, lateral, and transverse views can be requested by specifying the option AP, LA, and TR, respectively. Arbitrary views can be obtained by entering the coordinates of three points defining the plane. When standard plans are specified, the three points defining the plane are calculated by the computer and listed with the LP command.
- CO Continue on to the calculation of the isodose plans.
- ← (Carriage return) Terminate the program without calculating any isodose distributions. All data currently in the program are saved until a new case is entered.

Conclusions

This treatment planning program is being used routinely for ^{131}I and ^{192}Ir implants. It has been found to be easy to use. For every implant both orthogonal and stereo films are exposed. Whichever pair is easier to evaluate is used for computer input. The orthogonal pair is used for adding anatomy to the isodose distributions. The automatic choices for isodose plans make selection easy and reduce treatment planning time both by reducing input time and by reducing errors resulting from incorrect specification of the desired plan. The program is easily extendable to other seeds, such as radon and gold, as the need arises.

SUMMARY

An interactive treatment planning program for the computation of dose distributions for ^{131}I and ^{192}Ir seed implants of up to 300 sources is described. The seed coordinates are entered using either the keyboard or a sonic digitizer from either orthogonal or stereo films. The program produces a dose matrix which can be displayed and plotted directly or combined with the output of other treatment planning programs to provide composite plans for treatments using multiple modalities. Seeds of different

activities can be combined within a single plan. The program uses two-letter mnemonics for specifying the available options among which are commands for choosing standard antero-posterior, lateral and transverse plans.

ACKNOWLEDGEMENTS

These investigations were supported in part by U.S. Public Health Service Grants Ca 16127, CA 14052 and CA 21074 from the National Cancer Institute, DHEW.

REFERENCES

- ANDERSON I J. Dosimetry for interstitial radiotherapy. In: Handbook of interstitial brachytherapy, p. 87. Edited by B. S. Hilaris. Publishing Science Group Inc. Acton, Mass. 1975.
- HILARIS B. S. Techniques of interstitial and intracavitary radiation. Cancer 23 (1968) 745.
- Interstitial radiation with iodine 125. Panmineral 18 (1976) 28.
- KIM J. H. and TOKITA N. Low energy radionuclides for permanent interstitial implantation. Amer. J. Roentgenol. 126 (1976) 171.
- MARTINI N., BATATA M. and BEATTIE E. J. Interstitial irradiation for unresectable carcinoma of the lung. Ann. thorac. Surg. 20 (1975) 491.
- WHITMORE W. F., BATATA M. A. and GRABSTADT H. Radiation therapy and pelvic node dissection in the management of cancer of the prostate. Amer. J. Roentgenol. 121 (1974) 832.
- KIM J. H. and HILARIS B. Iodine 125 source in interstitial tumor therapy—clinical and biological considerations. Amer. J. Roentgenol. 123 (1975) 163.
- STOVALL M. and SHAFER R. J. A review of computer techniques for dosimetry of interstitial and intracavitary radiotherapy. Computer Progr. Biomed. 9 (1977) 125.
- SYFED A. M. and FEDER B. H. Technique of after loading interstitial implants. Radiol. clin. 46 (1977) 48.

EFFECTS OF WASHING ON PHYTOHEMAGGLUTININ RESPONSIVENESS
OF LYMPHOCYTES FROM IRRADIATED PATIENTS

■ MATSUBARA J HORIUCHI H SHIBUYA and M S SASAKI

Immunity against malignancy is believed to be predominantly a cellular mechanism involving tumor cell destruction by lymphocytes of thymic origin (WATERS 1978). The responsiveness of lymphocytes to phytohemagglutinin (PHA) stimulation which is related to delayed hypersensitivity decreases with ionizing radiation (BARAL & BLOMGREN 1976 RICKINSON & ILBERY 1971 STEWART & EREZ 1976). This phenomenon occurs in lymphocytes irradiated *in vitro* as well as in peripheral lymphocytes obtained from patients being irradiated for malignant disease (BLOMGREN *et coll* 1974 JALAS & WASSERMAN 1974 HOPPE *et coll* 1977 ILBERY *et coll* 1971 ORDER 1977). In addition several authors have reported generally lowered PHA reactivity in patients with malignant tumors before the beginning of any kind of therapy (MANNICK *et coll* 1977 McLAUGHLIN & BROOKS 1974 VETTO *et coll* 1975).

Irradiated peripheral lymphocytes have been washed for analysing the PHA responsiveness and the results are now reported. MANNICK *et coll* observed that non PHA responding lymphocytes of patients with malignant tumors after washing with a culture medium and incubation with normal serum exhibited an improved level of transformation. Therefore the frequency of chromosomal aberrations afforded by washing was also recorded.

Materials and Methods

In 60 patients who received radiation therapy for malignant tumors by fractionated doses of ^{60}Co γ

rays the changes in peripheral lymphocyte count were recorded.

These patients had a variety of malignant tumors including carcinomas of the head neck lung and esophagus testicular tumors and malignant lymphomas. The patients (22 females 38 males) varied in age from 9 to 81 years with a mean age of 59. The tumor dose ranged from 30 Gy per 3 weeks to 75 Gy per 9 weeks. Absolute lymphocyte counts were determined by multiplying the peripheral white blood cell count by the percentage lymphocyte in the differential and were displayed in relative value against the initial counts. In 16 of the 60 cases chromosome analysis of the peripheral lymphocytes was performed at the completion of the radiation therapy.

This analysis was repeated one to three times in the following 9 weeks in order to evaluate the fluctuation of chromosome aberration frequencies.

In 4 other irradiated cases not included in the series of 60 cases (Table 1) in which all known carcinoma had been removed operatively before irradiation PHA response and chromosome aberration frequencies were recorded using both direct and wash methods as described in the following. These patients were selected to exclude the possible effect of existing carcinoma on the inhibition of PHA response.

The blood used for the *in vitro* experiments was obtained from healthy young donors. An aliquot of the blood was irradiated in plastic tubes at room temperature by tele cobalt unit to a total dose of

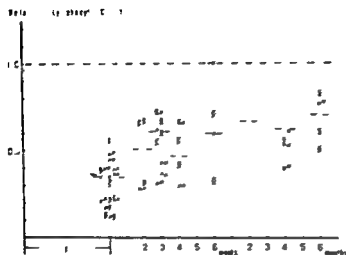


Fig. 1 Lymphocyte counts after irradiation in relation to pre-irradiation value

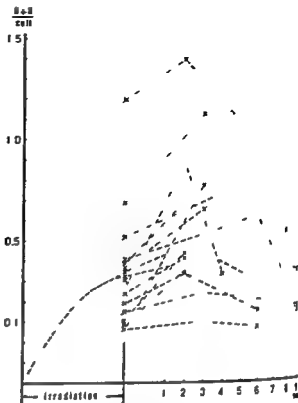


Fig. 2 Frequency of chromosome aberrations in peripheral blood lymphocytes after end of irradiation

either 4 Gy or 6 Gy using a dose rate of 0.82 Gy/min. For the determination of PHA responsiveness and chromosome aberration frequencies, lymphocyte containing blood was cultured in the medium RPMI 1640 supplemented with 20 per cent of fetal calf serum (Gibco) and 3 per cent of PHA M (Gibco). Colcemid ($0.5 \mu\text{g/ml}$) and thymidine ($5 \times 10^{-3} \text{ M}$) were also added to all cultures at 24 hours of incubation.

The cultures established from the irradiated cases as well as those from the healthy donors without irradiation were harvested at varying times in culture. The effect of washing with culture medium was recorded by comparing the results obtained from direct cultures with those from cells washed and suspended in culture medium for 18 hours before incubation.

Chromosome preparations were made according to

Table I
Summary of clinical data and sampling time

Case No.	Blood sampling	Interval between end of therapy and examination	Age and sex	Disease	Radiation dose
1	a	Immediately after the therapy	27 M	Testicular tumor	30 Gy in opposing fields
2	b	24 hours	42 M	Testicular tumor	33 Gy in opposing f. lds
3	c	4 days	45 F	Carcinoma of uterine cervix	47 Gy in opposing f. lds
4	d	31 days	45 M	Testicular tumor	45 Gy in opposing fields
	e	11 months	45 M	Testicular tumor	45 Gy in opposing f. lds

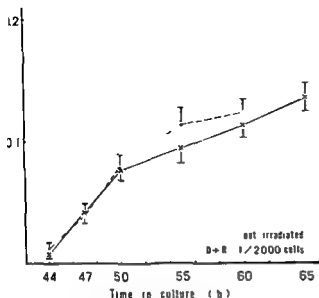


Fig 3 Mitotic index in cultures of non irradiated lymphocytes as a function of time in culture. Direct culture (—) Culture of lymphocytes after washing (---)

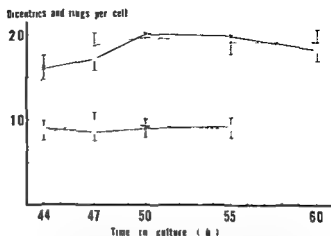


Fig 5 Frequency of chromosome aberrations in cultures of lymphocytes irradiated in vitro after 6 Gy (upper curves) and 4 Gy (lower curves). Direct culture (—) Culture after washing (---)

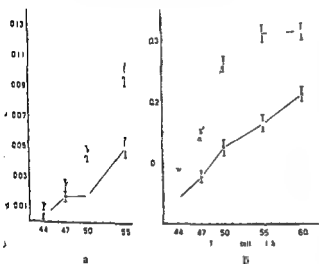


Fig 4 Mitotic index in cultures of lymphocytes irradiated in vitro after a) 4 Gy and b) 6 Gy. Direct culture (—) Culture after washing (---)

harvested at 55 hours of incubation and made by direct and wash methods respectively. At other incubation times 200 mitoses were analysed in each series. For the evaluation of PHA responsiveness mitotic index was determined on the basis of 2500 mononuclear cells.

Results

The changes in the peripheral lymphocyte count following the radiation therapy appear in Fig 1. The number of lymphocytes at the end of irradiation usually the minimum value was approximately 35 per cent of the initial count though the value varied possibly due to differences in radiation dose and field. The decrease in lymphocyte count was followed by a slight recovery usually occurring within about 3 weeks of the end of radiation therapy. Further improvement was found to be slight.

The chromosome analyses made in 16 of the 60 patients revealed a temporary rise in aberration frequency 2 to 3 weeks after the end of irradiation and a gradual decrease thereafter (Fig 2). The coincidence of a slight recovery of peripheral lymphocyte count with the rise in chromosome aberration frequency may be a reflection of a temporary replenishment of blood lymphocytes by previously irradiated lymphocytes stored in the extravascular system. The radiation induced delay in mitotic pro-

to the standard procedure which included hypotonic treatment with 0.9 per cent sodium citrate fixation in 3:1 methanol:acetic acid and flame drying. The slides were stained with Giemsa and analysed for mitotic indices and chromosome aberration yields. One hundred mitoses were then examined for the presence of dicentric rings, abnormal monacentrics and acentric fragments. In 2 cases with poor PHA response only 62 and 72 mitoses were analysed. In non irradiated cases 1000 mitoses were examined in 10 preparations.

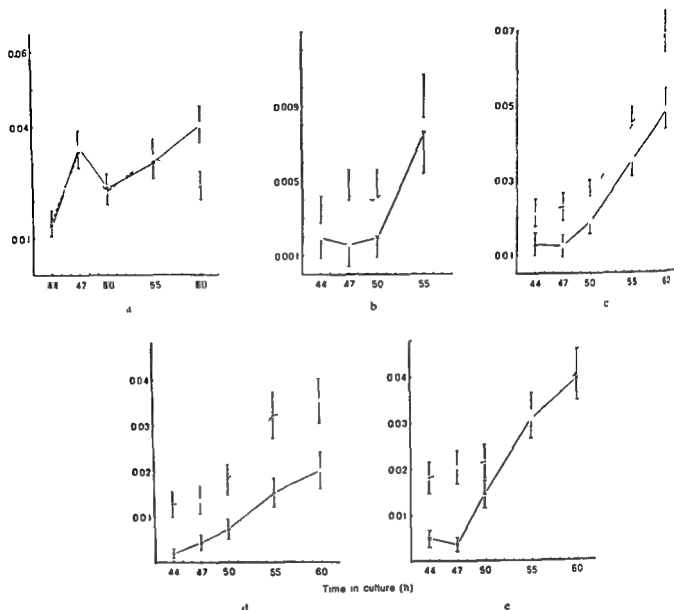


Fig. 6 Mitotic index in cultures of lymphocytes from patients receiving irradiation a) immediately after completion of therapy

b) after 74 hours c) after 4 days d) after 31 days and e) after 6 months Direct culture (—) Culture after washing (---)

gression and hyporesponsiveness to PHA may also influence the chromosomal aberration yields

The PHA reactivity of non irradiated peripheral lymphocytes was not significantly different between direct and wash methods as determined by the change in mitotic index against culture time (Fig. 3). However lymphocytes irradiated with 4 Gy in vitro showed higher mitotic indices in washed condition than in direct cultures (Fig. 4a). The increase was even more apparent in lymphocytes irradiated with 6 Gy in vitro (Fig. 4b).

Chromosome analysis in non irradiated lymphocytes using the direct method and harvested at 55 hours showed only one dicentric chromosome and

no dicentric was found in culture made after washing. The yields of dicentrics and rings in lymphocytes irradiated in vitro with 4 or 6 Gy showed no change in the frequency of chromosome aberrations with or without washing (Table 2, Fig. 5).

As for the PHA responsiveness of lymphocytes from patients receiving radiation therapy, the peripheral lymphocytes taken immediately after the completion of irradiation showed no improvement in PHA responsiveness by washing (Fig. 6).

An increase in mitotic indices caused by washing became evident in the lymphocytes obtained 4 or more days after the end of radiation therapy. The increase in mitotic index associated with washing

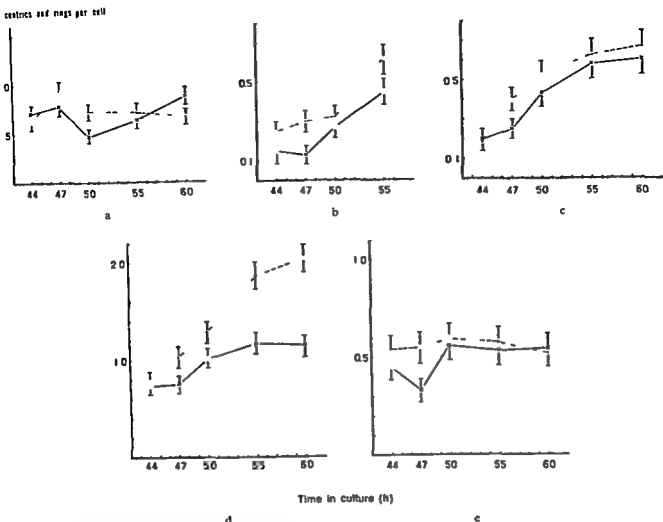


Fig 7 Frequency of chromosome aberrations in cultures of lymphocytes from patients receiving irradiation a) Immediately after completion of irradiation b) after 24 hours c) after 4 days

d) after 11 days and e) after 6 months Direct culture (—) Culture after washing (---)

was most remarkable in lymphocytes obtained 31 days after the end of irradiation (Fig 6). However, the lymphocytes taken from the patients 6 months after the end of radiation therapy showed only slight hyporeactivity in unwashed condition.

The changes in chromosome aberration yields against the time in culture are presented in Fig 7. The aberration frequency increased with the increase in culture time, particularly in cultures with improvement of the mitotic index by washing (Table 3). Fig 7) Washing of lymphocytes showed a further increase in chromosomal aberration yields which were still culture time dependent. The frequency of dicentric and rings among the cells with unstable chromosome aberrations, as expressed by Qdr values, also increased with the prolongation of incubation time (Table 3).

Discussion

The peripheral lymphocyte is one of the cells most sensitive to radiation. Its number decreases rapidly with increase in radiation dose (BARAL et coll 1977; BENJAMIN et coll 1978; BUCKTON et coll 1967; KWAN & NORMAN 1977; PROSSER 1976). The reduced number of lymphocytes caused by radiation is slow to recover and hardly resumes the pre-irradiated value even after one year (HOPPE et coll 1978; RUBIN & CASARETT 1968). In a report of patients who were irradiated for seminoma, the lymphocyte count only began to approach normal levels at 5 years after irradiation (HEIER et coll 1975). The most remarkable reduction of lymphocyte number is observed when large blood vessels are included within the radiation fields (CHEE et coll 1974), indicating that the lymphopenia is primarily due to the

Table 2

Frequencies of chromosome aberrations in cultures of lymphocytes irradiated in vitro

		Chromosome aberrations at indicated incubation time (h)							
Time in culture		44		47		50		55	
Types of culture		Direct	Wash	Direct	Wash	Direct	Wash	Direct	Wash
No irradiation	D+R/cell	0/200	0/200	0/200	0/200	0/200	0/200	1/1 000	0/1 000
In vitro irradiation	4 Gy D+R/cell	74/100	84/100	86/100	99/100	90/100	87/100	9/100	9/100
		0.90 ± 0.10	0.84 ± 0.09	0.86 ± 0.09	0.99 ± 0.10	0.90 ± 0.09	0.87 ± 0.09	0.97 ± 0.10	0.97 ± 0.10
	X _i Cu-cells	64	76	73	75	53	70	69	68
	Qdr ^d	1 16	1 11	1 18	1 32	1 38	1 24	1 33	1 30
	6 Gy D+R/cell	160/100	163/100	171/100	183/100	201/100	196/100	197/100	190/100
		1.60 ± 0.13	1.63 ± 0.13	1.71 ± 0.13	1.88 ± 0.13	2.01 ± 0.14	1.96 ± 0.14	1.97 ± 0.14	1.97 ± 0.14
	X _i Cu-cells	90	92	95	93	97	95	94	9
	Qdr ^d	1 78	1 77	1 8	2 02	2 07	2 06	2 10	2 0

No of dicentric plus rings/No of analyzed cells

* Mean \pm standard error

No of cells with unstable chromosomal aberrations which have not attempted a cell division since irradiation

* No of dicentric plus rings/X_iCu-cells

Table 3

Frequencies of chromosome aberrations in cultures of lymphocytes obtained from patients receiving irradiation

Blood sampling	Interval between end of therapy and examination	Radiation induced aberrations	Chromosome aberrations at indicated incubation time (h)					
			44		47		50	
			Direct	Wash	Direct	Wash	Direct	Wash
a	Immediately after the therapy	D+R/cell	71/100	62/100	78/100	94/100	49/100	77/100
			0.71 ± 0.08	0.62 ± 0.08	0.78 ± 0.09	0.94 ± 0.10	0.48 ± 0.07	0.77 ± 0.08
		X _i Cu-cells	36	36	33	49	77	40
		Qdr ^d	1 97 ^a	1 72	2 36	1 92	1 78	1 8
b	24 hours	D+R/cell	15/100	25/100	9/72	30/100	17/67	0/100
			0.15 ± 0.04	0.25 ± 0.05	0.13 ± 0.04	0.30 ± 0.06	0.27 ± 0.07	0
		X _i Cu-cells	20	18	18	27	19	18
		Qdr	0 75	1 39	0 64	1 11	0 89	1 14
c	4 days	D+R/cell	20/100	18/100	25/100	52/100	43/100	0/100
			0.20 ± 0.05	0.18 ± 0.04	0.25 ± 0.05	0.40 ± 0.07	0.43 ± 0.07	0
		X _i Cu-cells	16	11	20	32	37	16
		Qdr	1 25	1 64	1 25	1 63	1 34	1 4
d	31 days	D+R/cell	74/100	79/100	75/100	103/100	102/100	100/100
			0.74 ± 0.09	0.79 ± 0.09	0.75 ± 0.09	1.03 ± 0.11	1.02 ± 0.10	1.00 ± 0.10
		X _i Cu-cells	31	31	30	36	47	40
		Qdr	2 39	2 55	2 50	2 86	2 43	2 4
e	6 months	D+R/cell	45/100	54/100	33/100	55/100	56/100	0/100
			0.45 ± 0.07	0.54 ± 0.07	0.41 ± 0.06	0.55 ± 0.07	0.56 ± 0.07	0
		X _i Cu-cells	28	25	20	74	25	21
		Qdr	1 61	2 16	1 65	2 29	4	1

No of dicentric plus rings/No of analyzed cells

* Mean \pm standard error

No of cells with unstable chromosomal aberrations which have not attempted a cell division since irradiation

* No of dicentric plus rings/X_iCu-cells

Table 2 (cont.)

		65	
rect	Wash	Direct	Wash
00	0/100	0/00	-
	-	-	-
0/100	190/100	-	-
80 ± 0.13	1.90 ± 0.14		
	95		
88	2.00		

Table 3 (cont.)

		60	
rect	Wash	Direct	Wash
1/100	73/100	88/100	67/100
34 ± 0.08	0.73 ± 0.09	0.88 ± 0.09	0.67 ± 0.08
40	37	46	37
1.83	1.83	1.91	1.81
1/100	61/100	-	-
45 ± 0.07	0.61 ± 0.08		
41	41		
1.49	1.49		
1/100	67/100	60/100	66/100
58 ± 0.09	0.6 ± 0.08	0.60 ± 0.08	0.67 ± 0.08
41	37	40	47
1.68	1.68	1.5	1.57
7/100	186/100	113/100	703/100
17 ± 0.11	1.86 ± 0.14	1.13 ± 0.11	7.03 ± 0.14
53	53	43	58
3.51	3.51	2.63	3.5
1/100	57/100	54/100	52/100
53 ± 0.07	0.57 ± 0.08	0.54 ± 0.07	0.52 ± 0.07
23	23	4	-
2.48	2.48	2.25	2.3

killing of mature cells by radiation. Further patients with chest irradiation tend to have higher chromosomal aberration frequencies than those with abdominal irradiation (MATSUBARA et coll 1974).

In the present series the peripheral lymphocyte count in irradiated patients did not completely recover to the pre irradiated value even 6 months after the completion of irradiation though slight recovery was evident within 3 weeks.

The chromosome aberration yields in lymphocytes of irradiated patients generally increases with increase in accumulated radiation dose (BUCKTON et coll MELLO et coll 1974). The temporary rise in the frequency of radiation induced chromosomal aberrations 2 to 3 weeks after the end of irradiation appears to correspond with the slight recovery of peripheral lymphocyte number. This observation may be explained by the release of lymphocytes from the extravascular system, which have more chromosomal aberrations due to previous irradiation.

In addition there may be other factors to be considered in this temporary rise such as suppressor substance in the serum of patients being irradiated, radiation injury of lymphocyte membrane and delay in mitotic progression. These factors were analysed by washing the lymphocytes with culture medium and culturing the lymphocytes for various incubation times.

No effect of the wash was detected in the culture of non irradiated lymphocytes. The lymphocytes irradiated in vitro and in vivo sampled more than 24 hours after the end of irradiation showed an improvement of PHA responsiveness i.e. mitotic index after washing.

However in the lymphocytes sampled immediately after the end of fractionated irradiation no distinct improvement could be identified. This observation may be due to either inadequate washing (MANNICK et coll) or fragility of the lymphocytes immediately after high dose irradiation (STEFFEN & MICHALOWSKI 1973).

It has been found that the PHA responsiveness of lymphocytes in patients with advanced malignant tumors is usually defective but is generally improved by washing (MANNICK et coll WHITEHEAD et coll 1976). This improvement of PHA responsiveness in the lymphocytes of irradiated patients after washing with culture medium may be due to the elimination of a suppressor substance in the serum.

At least 3 mechanisms have been considered concerning the origin of serum blocking factors (VETTO *et coll*) (1) classic immunologic enhancement by autologous gamma 2 antibody (2) blocking factor produced by antigen-antibody complexes and (3) effect of a variety of proteins, peptides or alpha globulin factors (MCLAUGHLIN *et coll*)

The possible influence of radiation toxins released into the medium by cell death can be discounted (ILBERY & RICKINSON 1971) since medium collected from leukocyte cultures up to 24 hours after their exposure to 20 Gy could not reduce the subsequent response to PHA of non irradiated lymphocytes from the same donor. Further BENJAMIN *et coll* have reported that no inhibitory factor is present in plasma from irradiated dogs and that the defect appears to reside in the irradiated lymphocytes themselves. In the present series peripheral lymphocytes irradiated *in vivo* recovered their PHA responsiveness following washing with culture medium. This restoration of PHA reactivity is associated with an increase in the frequency of radiation induced chromosome aberrations in locally irradiated patients.

The concurrent change in Qdr ratio (SASAKI 1971) in the chromosomally aberrant cells indicates that the increase in the aberration frequency and hence the increase in the PHA responsiveness is due to the recovery of PHA reactivity of the more heavily irradiated fraction of cells.

In the parallel experiments using *in vitro* irradiation PHA responsiveness is also improved by washing but not associated with an increase in the chromosomal aberration frequency. The reason for this phenomenon perhaps lies with the difference between marked inhomogeneous clinical irradiation and uniform irradiation in experiments *in vitro* (SHARPE 1969 MATSUBARA *et coll*).

Mitotic progression in the present experiments was remarkably delayed in the lymphocytes of irradiated patients as evaluated by the chromosomal analysis. HEDDLE *et coll* (1967) were the first to report such radiation induced mitotic delay in lymphocyte culture. SHARPE using a mixed culture technique reported that an apparent peak existed in the aberration yields which was delayed by increasing the radiation dose. At 400 Gy of *in vitro* irradiation the duration of delay is reported to be in the order of a few hours.

In addition it has been shown that at a higher dose the delay is greater (RICKINSON & ILBERY)

and that the cells carrying dicentric (LLOID *et coll* 1977 STEFFEN & MICHALOWSKI) are more delayed in their rate of progress through the first cell cycle than irradiated but aberration free cells. In the present series marked delay in mitotic progression was found in the lymphocytes of irradiated patients especially in the lymphocytes with a high Qdr value.

All of these findings indicate that the defect in lymphocyte activation in patients receiving postoperative radiation therapy and in whom all malignant tumors are removed is caused by some reversible change in the lymphocyte membrane directly associated with the radiation exposure rather than by a radiation induced suppressor substance acting on the lymphocyte membrane. Glycoproteins in the cell membrane operate as receptors for various hormones and antigens. Their sugar portions contribute to the negative charge of the cell membrane (MARIKOVSKY *et coll* 1966 WEISS & SJOKS 1967 SATO *et coll* (1977) have shown that roentgen radiation can in fact induce a translocation of hyaluronic acid one of the carbohydrates in the membrane from the peripheral zone into the deeper zone. The reversal shift occurs in the recovery period. Disappearance of the effect of washing in lymphocytes obtained months after radiation therapy may be due to the recovery of the injured lymphocyte membrane and reflect a kind of *in vivo* washing.

Previous clinical experience has shown that progression of malignant tumors occasionally occurs following special hemodialysis (PARSONS *et coll* 1974). It would be of great value to determine whether such a phenomenon also occurs by washing induced restoration of PHA responsiveness of irradiated lymphocytes.

SUMMARY

The peripheral lymphocytes from irradiated patients generally have a reduced capability to respond to phytohemagglutinin (PHA). Whether a relationship existed between PHA responsiveness and chromosome aberration frequencies was examined by washing the lymphocytes with culture medium. The results indicate that the defect in lymphocyte activation in patients receiving radiation therapy was caused by some reversible changes in the lymphocyte membrane directly associated with radiation exposure rather than by a radiation induced suppressor substance secondarily acting on the lymphocyte membrane.

ACKNOWLEDGEMENTS

This investigation was supported by a Japanese Scientific research grant.

REFERENCES

- ARAL E and BLOMGREN H Response of human lymphocytes to mitogenic stimuli after irradiation in vitro *Acta radiol Ther Phys Biol* 15 (1976) 149
- BLOMGREN H, ENHORN N, LAX I and JUHLIN I Effect of radiation therapy on the mitogenic response of in vitro irradiated human lymphocytes to phytohemagglutinin *Acta radiol Ther Phys Biol* 16 (1977) 266
- BENJAMIN S A, HAHN F F and BOECKER B B Effects of chronic pulmonary irradiation on peripheral lymphocytes and their function in the dog *Radiat Res* 75 (1978) 121
- BLOMGREN H, WASSERMAN J and LITTBAND B Blood lymphocytes after radiation therapy of carcinoma of prostate and urinary bladder *Acta radiol Ther Phys Biol* 13 (1974) 357
- GLAS U, MELÉN B and WASSERMAN J Blood lymphocytes after radiation therapy of mammary carcinoma *Acta radiol Ther Phys Biol* 13 (1974) 185
- BUCKTON K E, COURT BROWN W M and SMITH P G Lymphocyte survival in man treated with X rays for ankylosing spondylitis *Nature* 214 (1967) 470
- LANGLANDS A B and SMITH P G Chromosome aberrations following partial and whole body X irradiation in man: Dose response relationships *In* Human radiation cytogenetics p 122 Edited by H J Evans W M Court Brown and A S Mclean North Holland Amsterdam 1967
- CHEE C A, ILBERY P L T and RICKINSON A B Depression of lymphocyte replicating ability in radiotherapy patients *Brit J Radiol* 47 (1974) 37
- GLAS U and WASSERMAN J Effect of radiation treatment on cell mediated immune response in carcinoma of the breast *Acta radiol Ther Phys Biol* 13 (1974) 83
- HEDDLE J A, EVANS H J and SCOTT D Sampling time and the complexity of the human leukocyte culture system *In* Human radiation cytogenetics p 6 Edited by H J Evans W M Court Brown and A S Mclean North Holland Amsterdam 1967
- HEIER H E, CHRISTENSEN I, FROLAND S S and ENGSEST A Early and late effects of irradiation for seminoma testis on the number of blood lymphocytes and their B and T subpopulations *Lymphology* 8 (1975) 69
- HOPPE R T, FLAKS Z Y, STROBER S and KAPLAN H S The long term effects of radiation on T and B lymphocytes in the peripheral blood after regional irradiation *Cancer* 40 (1977) 2071
- ILBERY P L T and RICKINSON A B Radiation damage to lymphocytes: Its expression in the kinetics of short term culture *In* Symposium on biological aspects of radiation quality Lucas Heights Australia 1971 (IAEA proc ser SM 145/41 p 297) IAEA Vienna 1971
- and THURM C E Blood lymphocyte replicating ability as a measurement of radiation dosage *Brit J Radiol* 44 (1971) 834
- KHAN D K and NORMAN A R Age of human lymphocytes and thymocytes *Radiat Res* 69 (1977) 143
- LOLLO D C, DOLPHIN G W, PLURROTT R J and TIPPER P A The effect of X ray induced mitotic delay on chromosome aberration yields in human lymphocytes *Mutation Res* 42 (1977) 401
- MANNICK J A, CONSTANTIN M, PARDDRIDGE D, SAPHOROSCHETZ I and BADGER A Improvement of phytohemagglutinin responsiveness of lymphocytes from cancer patients after washing in vitro *Cancer Res* 37 (1977) 3066
- MARIKOVSKY Y, DANON D and KATCHALSKY A Agglutination by polylysine of young and old red blood cells *Biochim biophys Acta* 124 (1966) 154
- MATSUBARA S, SASAKI M and ADACHI T Dose-response relationship of lymphocyte chromosome aberrations in locally irradiated persons *J Radiat Res* 15 (1974) 189
- MCLAUGHLIN III A P and BROOKS J B A plasma factor inhibiting lymphocyte reactivity in urologic cancer patients *J Urol* 112 (1974) 366
- MELLO R S, KWAN D and NORMAN A Chromosome aberrations and T-cell survival in human lymphocytes *Radiat Res* 60 (1974) 482
- ORDER S E The effects of therapeutic irradiation on lymphocytes and immunity *Cancer* 39 (1977) 737
- PARSONS F M, EDWARDS G F, ANDERSON C K, AHMAD S, CLARK P B, HETHERINGTON C and YOUNG A Regression of malignant tumors in magnesium and potassium depletion induced by diet and haemodialysis *Lancet* 16 (1974) 243
- PROSSER J S Survival of human T and B lymphocytes after X irradiation *Int J Radiat Biol* 30 (1976) 459
- RICKINSON A B and ILBERY P L T The effect of radiation upon lymphocyte response to PHA *Cell Tissue Kinet* 4 (1971) 549
- RUBIN P and CASARETT G W Clinical radiation pathology Volume II p 78 Saunders Philadelphia 1968
- SASAKI M S Radiation induced chromosome aberrations in lymphocytes: Possible biological dosimeter in man *In* Biological aspects of radiation protection p 81 Edited by T Sugahara and H Igarashi Shoin Tokyo 1971
- SATO C, KOHMA K and NISHIZAWA K Translocation of hyaluronic acid in cell surface of cultured mammalian cells after X irradiation and its recovery by added adenosine triphosphate *Biochim biophys Acta* 470 (1977) 446
- SHARPE H B Pitfalls in the use of chromosome aberration analysis for biological radiation dosimetry *Brit J Radiol* 42 (1969) 943
- STEFFEN J and MICHALOWSKI A Heterogeneous chromosomal radiosensitivity of phytohemagglutinin-stimulated human blood lymphocytes in culture *Mutation Res* 17 (1973) 367
- STEWART C C and PEREZ C A Effect of irradiation on immune responses *Radiology* 118 (1976) 201
- VETTO R M, BURGER D R, VANDENBARK A A and VOLTE J Influence of serum blocking factors on cancer patients undergoing immunotherapy *Amer J Surg* 130 (1975) 237
- WATERS H (Editor) The handbook of cancer immunology

- g. Volume I Basic cancer related immunology Garland STPM Press New York 1978
- WEISS L and SINKS L F The electrokinetic surfaces of human cells of lymphoid origin and their ribonuclease susceptibility Cancer Res 30 (1970) 90
- WHITEHEAD R II THATCHER J TEASDALE C and ROBERTS G P T and B lymphocytes in breast cancer stage relationship and abrogation of T lymphocyte depression by enzyme treatment in vitro Lancet (1976) 330

**Procedures in External Radiation
Therapy Dosimetry with Electron and Photon
Beams with Maximum Energies
Between 1 and 50 MeV**

**Recommendations by
the Nordic Association of Clinical Physics (NACP)**

These recommendations have been produced by the Swedish Association of Radiation Physics and the Nordic Association of Clinical Physics aided by a large number of their members. The members of the Topic Group were H. Svensson (chairman), L. Lindborg (secretary), K. A. Johansson and N. Ulso. Valuable criticism and aid is gratefully acknowledged. P. Almond, A. Brahme, J. Flatby, D. Harder, O. Mattsson, I. Uotila and the Ph.D. students and teachers from the Radiation Physics Department in Linköping.

The meetings of the working party were supported both by the Swedish and Danish Cancer Society.

CONTENTS

INTRODUCTION	58
ENERGY DETERMINATION AT ACCELERATORS	59
Electron beams	59
Photon beams	61
GEOMETRIC CONSIDERATIONS	63
Beam alignment	63
Depth of reference plane	64
Uniformity of the beam	64
DETERMINATION OF ABSORBED DOSE AT REFERENCE POINTS	65
Ionization chambers and electrometers	65
Calibration at national standards laboratories	66
Derivation of absorbed dose ionization chamber factor	67
Absorbed dose determination at the reference point	67
Illustrative example	69
DETERMINATION OF ABSORBED DOSE AT ANY POINT	71
Beam axis absorbed dose distribution	71
Iso absorbed dose distributions	73
MAINTENANCE PROGRAM FOR DOSIMETRY OF THERAPY UNITS	74
Radiation beam alignment checks	74
Absorbed dose monitor checks	75
Radiation energy constancy checks	75
Radiation beam uniformity checks	75
CHECK OF ABSORBED DOSE GIVEN TO THE PATIENT	75
Absorbed dose monitoring systems	75
Absorbed dose measurements on patients	76
APPENDIX	76
REFERENCES	77

Introduction

The International Commission on Radiation Units and Measurements (ICRU) has published general recommendations on dosimetry procedures for photons (ICRU 1969) and for electrons (ICRU 1972). These should preferably be supplemented by national or regional suggestions covering practical details of routine dosimetry procedures and taking into account the particular requirements and provisions of the country and region. Local recommendations have been prepared for the United Kingdom (HPA 1969 1971 1975), the USA (SCRAD 1966 1971 AAPM 1975), West Germany (DIN 6809 1976 DIN 6800 1975 a, b) and the Nordic countries (NACP 1972).

The present report contains a revised Nordic protocol. Several reasons have motivated this revision. After publication of the first protocol several reports have been published giving new data on various effects which can change the factors used with ionization chamber dosimetry. The SI units for the radiologic quantities should be applied. Another important reason is that the former Nordic recommendations were mainly based on investigations with betatrons, while within the Nordic countries now several other kinds of accelerators are used (standing wave and travelling wave linear accelerators and microtrons) with usually different properties which have to be considered. Improved concepts for stating beam quality and beam uniformity etc. are therefore introduced in the present report. Similar revisions are being carried out by the ICRU and the AAPM.

Differences in the absorbed dose determination using the methods given in NACP (1972) compared to methods given in the present protocol can be as large as 5 per cent in extreme cases. It is recommended that the new protocol will be adapted at various centres as soon as new calibrations of ionization chambers have been achieved.

In the present report *shall* means compulsory for compliance with this report and *should* means strongly recommended.

These recommendations do not include the complete procedures for dosimetry of electron beams in the range of 1 to 10 MeV mean electron energy

at the phantom surface where plane parallel chambers shall be used. Such procedures will be planned in a supplement to this protocol to be published in the near future.

The aim of the recommendations is to give hospital physicists a code of practice to be followed at radiation therapy centres in Denmark, Iceland, Norway and Sweden so as to secure conformity in dosimetry procedures. They cover tests and measurements both when dosimetry is first performed with a new therapy apparatus and the continuous supervision necessary to ensure that the dosimetry is properly employed in radiotherapy. In weighting high accuracy and the strictness against practical usefulness, the latter has been given more emphasis so that the procedure may be performed easily at all therapy centres.

Principal features. The dosimetric method using air ionization chambers is still recommended, linking the national standard to the local reference, mainly in view of their general availability, accuracy and precision. For the radiation quantities concerned here the ionization chambers shall be calibrated at national radiation standards laboratories in ^{60}Co - γ ray beams.

Since the formerly used C_{p} and C_{w} values only valid for air equivalent and water equivalent chamber walls respectively, a new procedure for the determination of absorbed dose is recommended. The procedure is based on a derived absorbed dose ionization chamber factor (N_D), the ratio between the mean absorbed dose to the ionization chamber cavity and the scale reading. N_D is derived from a calibration in air in a γ ray beam. The absorbed dose to water at the radiation qualities is then obtained as the product of meter readings, N_D , stopping power ratios and perturbation factors. Stopping power ratios and perturbation factors are both listed. The latter are for chambers of either air or water equivalent materials, the equivalence of concern being the ability of generating secondary electrons. Water equivalent materials are recommended for ionization chambers at least for reference measurements.

Table 1
Energy quantities for specifying radiation beams

Quantity	Use	Determination	Energy range
E	On accelerator console	$E = E_0 + \sum S_{01} \Delta Y_1$ eq (1)	1 MeV $\leq E \leq$ 50 MeV
E_0	Specification of absorbed dose distribution	$E_0 = C + C_2 R + C_3 R$ eq (2) $C = 0.72$ MeV $C_2 = 1.98$ MeV cm ⁻¹ $C_3 = 0.0025$ MeV cm ⁻²	1 MeV $\leq E \leq$ 50 MeV
E	A ₅ reference to dosimetric constants	$E_0 = C R_{50}$ eq (3) $C = 2.33$ MeV cm	5 MeV $\leq E_0 \leq$ 30 MeV

The increasing number of accelerators of different types and often quite different beam qualities necessitates a simple unified comparison of the quality of one beam with another. The main therapeutic and physical properties of the depth dose distribution of the beams are therefore characterized by new parameters not used in NACP (1972).

Energy determination at accelerators

A knowledge of the radiation quality is necessary because stopping power values and perturbation factors recommended here for ionization chamber dosimetry are energy dependent and because standardized depth dose tables may be used for accelerators similar in construction provided the energy is determined in a uniform manner (SVENSSON & HETTINGER 1971, SVENSSON 1971). Furthermore quality parameters may be desired for the comparison of one beam with another. Depending on the parameter of interest different energy quantities are recommended. For electron beams it is recommended to use a therapeutic range to describe the radiation quality in irradiation procedures.

Electron beams

Energy quantities. The intrinsic accelerator beam of the electron beam just before the exit window of the accelerator but after beam handling magnets and energy defining slits has a certain energy distribution. This can be characterized by its maximum energy (E_{m0}), its most probable energy E_{p0} , its mean energy E and its energy spread Γ_0 , the index 0 stands for accelerator. As the beam passes through the exit window and different materials from the exit window to the phantom surface the energy will decrease and the energy spread increase.

Therefore analogous energy quantities can be defined for the phantom surface index z and for any depth x in the phantom index z . These different electron energy quantities are recommended and are summarized in Table 1 and Fig. 1. Their principal uses are:

E_{p0} (the most probable energy in front of the accelerator window). For a given accelerator beam setting different beam scattering foils or decelerators are often used. However the energy instruments are usually constructed to give a measure of the electron energy of the intrinsic beam. Therefore it is recommended that the energy indication meter and the energy selection setting on the console desk be calibrated in an energy quantity which is independent of the materials in the beam. E_{p0} should be the energy quantity to use for this purpose.

E_{p0} (the most probable energy at the phantom surface). To indicate the energy of an absorbed dose distribution E_{p0} shall be used. The reason is that this energy quantity is well related to the practical range R_p which generally is used for energy determination. E_{p0} is in most accelerator facilities 1 to 2 MeV lower than E_{p0} for energies below some 20 MeV. This difference may increase with energy.

\bar{E}_0 (the mean energy at the phantom surface). In absorbed dose measurements with an ionization chamber the relevant stopping power ratios and perturbation factors must be known. These are in this protocol given as a function of E_0 and the depth of the chamber in the phantom (Tables 5-7). [In NACP (1972) the absorbed dose conversion factors (C_E) were correlated to the mean energy at a phantom depth, a quantity estimated from the relation

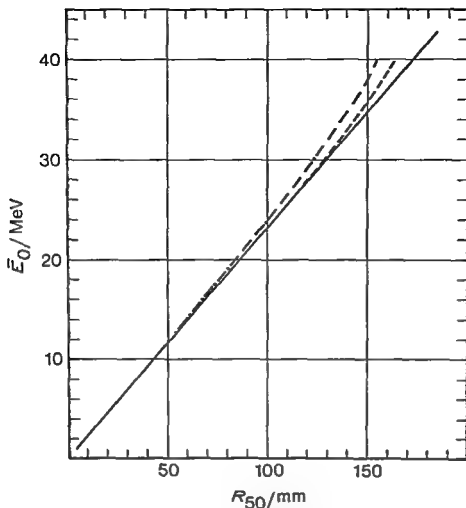


Fig 3 The relation between R_{50} and E for large field sizes. The solid line is valid for R_{50} determined from beam axis depth absorbed dose curves with SSD= ∞ the broken line for beam axis

depth absorbed dose curves with SSD=1 m and the dash-dotted line for beam axis depth ionization curves with SSD=1 m

Above that energy inverse square law corrections should be performed. R_{50} should be measured with large field sizes and should be determined from a depth absorbed dose curve but may be evaluated from a depth ionization curve and the graph in Fig 3.

$E_{p,2}$ should be calculated according to eq (1) in Table 1. The calculation means that the energy

losses of the electrons in all scattering materials in the radiation beam e.g. window scattering foils transmission chambers and air must be added to the electron energy $E_{p,2}$. The thickness of the various materials $\Delta\lambda_1$ which the beam passes from the inner side of the tube window to the phantom surface must be known as well as the collision stopping power of each material $s_{coll,1}$. Materials in the beam very near the phantom surface should if possible be removed in the measurements of $E_{p,2}$ as eq (1) gives an incorrect estimate of $E_{p,2}$ with materials near the phantom surface.

Photon beams

Energy determinations For a proper choice of stopping power ratios and perturbation factors in photon beams a measure of the photon beam quality can be estimated from depth ionization measure

Table 2
Some characteristics of phantom materials

Phantom material	Composition	■ g cm	$\left(\frac{Z}{A}\right)_{\text{eff}}$	■
Water	H O	1	0.555	1.000
Polystyrene	C H	1.05	0.518	1.018
Perspex	C H O ₂	1.18	0.540	1.148
A 150	see ICRU (1977)	1.1	0.548	1.106

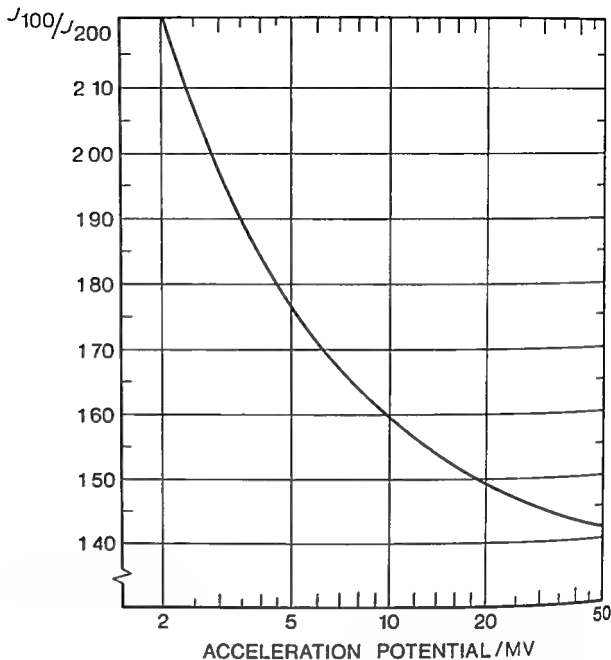


Fig 4 The ratio J_{100}/J_{200} ($=D_{100}/D_{200}$) as a function of the accelerating potential ($h\nu_m$) is shown for a SSD=1 m and a field size of 100 mm \times 100 mm. For accelerators with the same accelerating potential the ratios differ from beams with different targets and flattening filters and are as a rule smaller for beta

trons than for linear accelerators and microtrons. All ratios J_{100}/J_{200} above 20 MV are from betatrons while those below 20 MV are taken from linear accelerators. J_{100}/J_{200} is recommended as method for (s_{100}) .

ments (BRAHME & SVENSSON 1979). The ionization at the depth of 100 mm and 200 mm in a water phantom is measured for a field size of 100 mm \times 100 mm and an SSD of 1 m and the ratio J_{100}/J_{200} determined. The ratio is stronger related to the mean photon energy than the maximum photon energy. Therefore if the ratio is calculated from published data and plotted against the maximum photon energy a rather large spread is found (Fig 4). The J_{100}/J_{200} method is recommended for estimation of

the photon beam quality rather than measurement of the half value depth R_{50} as the values of R_{50} depend upon the contamination of electrons in the peak absorbed dose of the photon depth dose curve.

The maximum photon energy $h\nu_m$ in the photon beam can be estimated with two different methods. When both photon and electron beams are available from the same accelerator the maximum photon energy may within 1 or 2 MeV be approximately $E_{\beta m}$. The energy meter on the accelerator is used

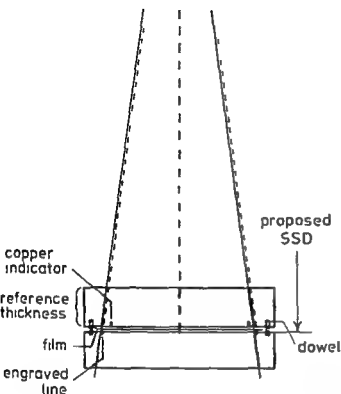
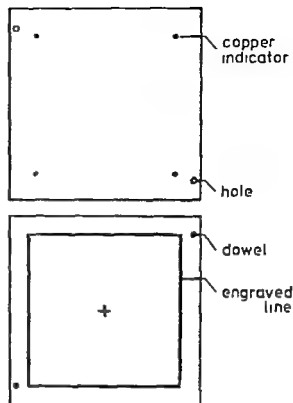


Fig 3 Polystyrene phantom to be used for beam alignment check. A small misalignment is indicated in the figure as the solid and broken lines do not coincide. Light beam — Radiation beam — Beam axis



designed to give a measure of the electron energy before the exit window and photon target. The meter can therefore be calibrated against E_p for the electron beams (see page 5). Alternatively a method based on (γ, n) threshold measurements may be used (NACP 1972). This method makes a direct calibration of the energy instrument in the maximum photon energy $h\nu_m$ possible. The maximum photon energy has conventionally been used to specify depth dose distributions.

Recent investigations have shown that the shape of the depth absorbed dose curves and the mean energy for photon beams may be more dependent on the construction of target and flattening filter than on a change of a few megavolts in accelerating potential, i.e. in change of E_m (PODGORSK et coll 1975, NAHUM 1978). Therefore it is not considered necessary to carry out determinations of $h\nu_m$ using the methods mentioned, neither for the purpose of dosimetry nor for the specification of depth absorbed dose curves. The J_{100}/J_{200} method is uncertain for the estimate of the maximum photon beam energy but is well related to the mean photon energy and should therefore be used for dosimetric procedures. The ionization ratio is recommended

as a beam quality parameter for use in choosing the stopping power ratios necessary for ionization chamber dosimetry (Table 6).

Geometric considerations

The position of the radiation beam must be defined and indicated to be able to perform accurate radiation therapy. In this section some simple procedures are given. The discussions are limited to rectangular unmodified beams only, i.e. without wedges or blocks.

Beam alignment

The beam alignment shall be checked on the installation of a new therapy unit. The manufacturer should be held responsible for any adjustment required before the accelerator is handed over for routine treatments.

The proper alignment of the different types of beam axes (collimator rotation axis, geometric beam axis, radiation beam axis, and light beam axis as defined in the Appendix) shall be checked before the absorbed dose distributions are determined. Practical beam alignment procedures have been detailed in

Table 3
Depth of reference plane

Type of radiation	\bar{h}_m or E	Depth of reference plane
Photons	1 - < 10 MeV	40 mm
	10 - 50 MeV	100 mm
Electrons	1 - < 5 MeV	Peak absorbed dose
	5 - < 10 MeV	Peak absorbed dose or min 10 mm
	10 - < 20 MeV	Peak absorbed dose or min 20 mm
	20 - 50 MeV	Peak absorbed dose or min 30 mm

the case of electron and photon beams by HPA (1970) AAPM (1975) and may be consulted when the checks are carried out. Also the International Electrotechnical Commission (IEC) has a working group dealing with such procedures.

An alternative method to test the position of the light beam in comparison with the radiation beam appears in Fig. 5. The test should be carried out in the following order. The bottom sheet of the phantom is placed perpendicular to the collimator axis with the front surface at the SSD in use. The light beam size and position shall coincide with the engraved line on the bottom sheet. The numerical field size shall be noted. A film in a light tight cover is placed on the bottom block and a top block with 4 metal indicators is placed with care on the film with out moving the bottom block. The thickness of the top block should be approximately equal to the reference depth (Table 3). After irradiation 4 dots on the film are observed from the indicators. These dots should be used for comparison of the position of light and radiation beam. The uniformity index, the radiation field size and the physical penumbra may also be determined from the same film. The beam and the collimator axes shall agree within 2 mm and the position and size of the radiation beam and light beam shall agree within 2 mm at an SSD of about 1 m.

Depth of the reference plane

Recommended depths for the reference plane for various radiation qualities are given in Table 3. For electron beams the depth of the absorbed dose maximum is recommended as the reference plane instead of a fixed depth due to the peaked depth absorbed dose curves for some low energy electron beams. However for some accelerators and beam sizes the maximum absorbed dose can occur at very

small depths due to electrons scattered from the in-scatter collimators. In those cases the depth of the reference plane should be taken as the maximum values given in Table 3.

Uniformity of the beam

A useful measure of the beam uniformity is the uniformity index (see Appendix). This index should not exceed 0.80 in the reference plane for the absorbed dose at field sizes larger than 100 mm x 100 mm for both photon and electron beams. In addition, the beam uniformity should be such that the absorbed dose at any point in the reference plane should not exceed 103 per cent of that at the reference point. For accelerator beams the physical penumbra (see Appendix) shall not exceed 8 mm at an SSD of about 1 m. Some accelerators are overflattened at small phantom depths in order to achieve a good uniformity index at large depths. Then for any plane parallel to the reference plane the absorbed dose at an arbitrary point in that plane should not exceed 11 per cent of its value on the beam axis.

The radiation beams should as minimum requirements fulfil the values given but each hospital physicist should work for achieving as good beam uniformity as possible generally aiming at an absorbed dose variation within the target volume of less than ± 5 per cent (ICRU 1976).

The uniformity of the radiation field size and the physical penumbra may be measured in various ways. Photographic film in a polystyrene phantom offers the advantages of high spatial resolution, simplicity of handling, short irradiation times and above all the fact that it will lead to simultaneous recording of the entire radiation field. With some care the figures given for absorbed dose uniformity may be equated to the same figures obtained for net film blackening. A disadvantage of the film method is however that it is best used with an automatic or semi automatic density plotter. With the film method the beam uniformity should be investigated by means of photographic film in a light tight cover of regular thickness but not of a radiation fluorescent material. Industrially pre-wrapped film may be used. The film should be exposed in a polystyrene phantom (Fig. 5) and the absorbed dose should be about 1 to 2 Gy (a typical treatment absorbed dose) as to minimize the influence of initial perturbation in accelerator beams and of shutter movement in ^{60}Co beams. Before irradiation an acceleration should have been run under operating conditions for

sufficient period of time to effect a proper warm up. The relation between film blackening and the absorbed dose depends critically on the development procedure used. Parameters of importance are type of film and developer, development time and temperature. All these should be combined to produce a linear relationship between blackening and absorbed dose over a large range. The aperture diameter of the density reader should be selected to exert negligible influence on the spatial pattern recorded. The variation in film density on a homogeneously exposed film should be less than ± 2 per cent only. A few types of film seem to meet these requirements (RASSOW et coll 1969, DUTREIX 1976). The film density versus absorbed dose should be checked regularly and at least every time a film batch or developer is changed.

Alternatively a semi-conductor detector or a small ionization chamber (i.e. diameter of not more than 5 mm) could be used for traversing the beam in the reference plane in a water tank for gantry angles at 30° , 270° and possibly 0° from the vertical position. This method will produce higher precision than the film method provided that in connection with accelerators a monitor probe is used to correct for output fluctuations. The monitor probe should be placed either outside the region of interest (the 50 per cent isodose curve) or in the centre of the beam in which case the shadowing effect must be taken into account when evaluating the results. For other gantry angles the beam could be investigated by means of a polystyrene block with holes for the detector (NAYLOR & CHIVERALLS 1970). The absorbed dose distribution in the reference plane may then be equated to the ionization current distribution.

Determination of absorbed dose at reference points

The absorbed dose determination shall be made by air ionization chamber measurements performed by a qualified person. A new determination shall be made when new conditions of irradiation are employed. The determination shall be confirmed with an independent dosimetric method for instance calorimetry or ferrous sulphate dosimetry and even less accurate methods such as those based on solid state dosimetry may be used. Both the absorbed dose determination and confirmation shall be performed before irradiation of patients. In connection with

these absorbed dose determinations a constancy check procedure shall be incorporated.

Ionization chambers and electrometers

Reference instrument Each radiation therapy centre shall have at least one local reference (standard) ionization chamber together with an electrometer selected as a reference instrument for calibration of field ionization chambers and other dose meters. A reference chamber may also be used for the first calibration of a new therapy machine. However, due to good stability of modern electrometers a reference electrometer may be used also for other purposes. The reference chambers shall be calibrated at a standardizing laboratory particularly for ^{60}Co γ rays in air. The reference electrometer shall also be calibrated at a standardizing laboratory. The calibration is preferably carried out separately for the ionization chamber and the measuring assembly. The chamber should be re-calibrated at a standardizing laboratory at least once every 2 years. A longer interval can be acceptable for the electrometer if its stability can be checked in an independent way.

The response of the reference instrument (ionization chamber and electrometer) should be checked at least quarterly against a suitable radioactive source at the radiation therapy centre, e.g. ^{60}Co γ source (half life 5.27 ± 0.01 year). Any change in response of the reference instrument of more than 1 per cent revealed by the constancy checks should lead to a thorough investigation of instrument and subsequent re-calibration at the standardizing laboratory.

A cylindrical chamber with an air volume of 100 to 1000 mm³ should be used as reference ionization chamber. If the chamber is to be used for calibrations of field instruments under conditions other than those at the standardizing laboratory (for instance in a phantom) it is essential that the air volume diameter in the chamber is between 4 and 8 mm and has a length of less than 25 mm. The wall of the chamber should be homogeneous graphite or tissue/water or air equivalent material. The thickness of the graphite chamber wall should preferably be about 0.5 mm as the perturbation factors reported here have been determined with this wall thickness (ALMOND & SVENSSON 1977, JOHANSSON et coll 1977). The wall thickness is of less importance for water equivalent chambers. It is important that the material of the build up cap is the same as that of the wall. In order to avoid mistakes the build

should be clearly marked and shall belong to a certain ionization chamber. The central electrode should be made of the same material as the wall and should preferably not be too massive. Leakage current, radiation induced current and current generated in the stem must be negligible. The ratio between the ionization currents measured at positive and negative polarizing potential shall be checked and should be less than 1.005 for any radiation beam quality. This polarity effect increases with decreasing electron energy and should therefore for a cylindrical ionization chamber be determined at various depths in a phantom for a beam of about 10 MeV mean electron energy at the surface.

Field instruments. The ionization chamber used for the assessment of absorbed dose must fulfil certain requirements. A cylindrical ionization chamber should be used at all photon radiation qualities and at electron beams with a mean energy at the phantom surface E_0 above 10 MeV. A plane parallel chamber should be used at electron energies E_0 equal to or less than 10 MeV. A supplement of this protocol will treat the measurement procedure used for the plane parallel chamber.

The cylindrical field chamber should have dimensions like the reference chamber. Chambers with unknown wall, central electrode material and thickness or with a thin layer of inner conducting material should be subjected to a special response test. It should be calibrated against the reference chamber for all radiation qualities used. Leakage current, radiation induced current and current generated in the stem must be negligible.

The response of the field instrument should be checked at least quarterly against a suitable radioactive source or against the reference instrument. Any change in sensitivity of the field instrument of more than 1 per cent revealed by the constancy check should lead to a thorough investigation of the instrument and subsequent re-calibration against the reference instrument.

Ion recombination. The losses due to ion recombination are generally less than 1 per cent for continuous radiation or pulsed radiation of an absorbed dose to air in the chamber cavity per pulse of 1 mGy or less if the collection voltage is higher than 300 V for the type of chamber mentioned above. If the losses are less than 1 per cent, recombination correction will not always be necessary. Correction for recombination losses can be made either by calculation (BOAG 1966, ICRU 1964) or by measure-

ments (ICRU 1972). For pulsed radiation beams, the measured charge is plotted against the inverse of the polarizing voltage in the region of losses below 1 per cent. The released charge is determined by extrapolation in this plot to infinite polarizing voltage.

Electrometer. A precision low current measuring electrometer shall be used. The electrometer is usually based on a high gain amplifier with a low leakage current working either in a charge/voltage mode or in an integrating Townsend balance mode. A digital display is usually preferable. A solid state electrometer may be built with either field effect transistors (MAUDERLY & BRUNO 1966) or a varactor bridge input amplifier (JOHANSSON *et al.* 1972). The scale on the electrometer should preferably be marked in coulomb and ampere. The electrometer should have a good long term stability for a number of years.

The instruments shall be constructed so as to minimize the effect of external electrostatic, magnetic and electromagnetic fields. However, a strong electromagnetic field that exists in and around some accelerators may affect the reading of the instrument. Special attention is needed to ensure that this does not occur. However, large mistakes in the dosimetry due to these effects will be covered as 2 independent methods shall be used for absorbed dose determination at an accelerator.

Calibration at national standards laboratories

The local reference chamber and measuring electrometers shall be calibrated in national standards laboratories in a beam of ^{60}Co rays. The calibration of the ionization chamber shall be made in air at a distance of about 1 m from the source to the chamber centre and at a field size of about 100 mm² or 100 mm. The cylindrical chamber should have an additional cap of water or air equivalent material, chambers of water and air equivalent walls respectively to assure electron equilibrium. The thickness of wall and additional material should be $0.45 \pm 0.05 \text{ g cm}^{-2}$. If a perspex cap is used, it should be of the recommended materials and if this procedure is followed the systematic error introduced will be less than 1 per cent (ALMOND & SVENSSON 1971, JOHANSSON *et al.* 1977).

International recommendations now exist for primary standards laboratories to derive air kerma from exposure measurements using agreed conversion factors. The relation between air kerma (K_{air}) and exposure (X) is (ICRU 1971)

$$A_{air}(1-g) = W/e \quad (5)$$

where

g = the fraction of the energy of the secondary charged particles lost to bremsstrahlung in air (g is at ^{60}Co γ rays close to 0.4 per cent BOU TILLON 1977)

W/e = mean energy expended in air per ion pair formed and per electron charge (W/e is equal to 33.85 J C^{-1} ICRU (1979))

The laborious use of the SI units for exposure (C kg^{-1}) in some applications can then be overcome from the use of air kerma (unit Gy). The Nordic standards laboratories will be able to provide air kerma calibration factors (N_A) at ^{60}Co γ beams defined by

$$N_A = \frac{A_{air}}{M_c} \quad (6)$$

where

A_{air} = kerma in air at the centre of an ionization chamber in the absence of the chamber at the calibration radiation quality in Gy

M_c = meter reading at calibration corrected for temperature pressure humidity etc in C or div

(General re combination corrections should not be necessary to perform at the calibration in ^{60}Co γ beams with the types of chambers recommended in this protocol)

Derivation of absorbed dose ionization chamber factor

The mean absorbed dose to air D_{air} inside the air cavity of the ionization chamber has to be evaluated from the observed air kerma A_{air} if as suggested in this report the Bragg Gray relation is to be used for determination of absorbed dose to water. The following relation between D_{air} and A_{air} is used

$$D_{air} = A_{air}(1-g) k_{at} k_m \quad (7)$$

where

k_{at} = attenuation and scattering factor correcting for attenuation and scattering in the ionization chamber material at the calibration in the ^{60}Co - γ beam

Table 4

k_{at} and k_m values for cylindrical ionization chambers of sizes recommended in this protocol for different materials of the wall and cap combination. The values are expected to be dependent on the shape size and electrode design

Chamber wall and cap material	k_{at}	k_m
Air equivalent	0.990	1.000
Graphite	0.990	0.991
Tissue equivalent (A 150)	0.990	0.963

k_m = chamber material dependent factor correcting for the lack of air equivalence of the ionization chamber material

The correction factors k_{at} and k_m have been discussed extensively in the literature recently e.g. by ALMOND & SVENSSON (1977) and JOHANSSON et coll (1977)

The symbol A with various indices is often used instead of k_{at} and k_m different authors giving different meaning to the symbol. The factors recommended (Table 4) are those given by JOHANSSON et coll (1977)

The absorbed dose to air ionization chamber factor N_D is derived from eqs (6) and (7) as

$$N_D = \frac{D_{air}}{M_c} = N_A(1-g) k_{at} k_m \quad (8)$$

N_D could be derived for any ionization chamber but the values in Table 4 are only valid for chambers described on p. 11. N_D could be stated by national standards laboratories or be calculated at the hospitals. If given by standards laboratories the factors k_{at} and k_m must be clearly stated in the protocol (N_D is related to an exposure calibration factor (N_X) through the formula

$$N_D = N_X k_{at} k_m \frac{W}{e} k_1 \quad (9)$$

where

$$k_1 = 1.00 \text{ with } N_X \text{ in } \text{C kg}^{-1} \text{C}^{-1}$$

$$k_1 = 2.58 \cdot 10^{-4} \text{ with } N_X \text{ in } \text{R C}^{-1}$$

Absorbed dose determination at the reference point

For all photon radiation beams with maximum energies above 1 MeV and all electron radiation

Table 5

Recommended values of $(s_{w,1})$ and p for electron radiation at the reference point in a water phantom. The absorbed dose maximum is assumed to be at the minimum reference depths given in Table 3. The $(s_{w,air})$ shall be taken from Table 7 if dose maximum and therefore the reference point is situated at larger depths than given in Table 5. The p may be taken from Table 5 as it is not critically dependent on the depth.

E (MeV)	R_{10} (absorbed dose measure- ment) SSD=1 m (mm)	R_{10} (ionization measure- ment) SSD=1 m (mm)	Minimum reference depth (mm)	$(s_{w,1})$	p
1	3	3	2	1.144	-
2	7	7	4	1.137	-
3	12	12	6	1.122	-
4	16	16	8	1.108	-
5	21	21	10	1.097	-
6	25	25	10	1.078	-
7	30	30	10	1.061	-
8	34	34	10	1.048	-
9	38	38	10	1.036	-
10	43	43	10	1.053	(0.975)
12	51	51	20	1.033	0.980
14	60	59	20	1.018	0.985
16	68	67	20	1.006	0.985
18	78	76	20	0.997	0.990
20	86	84	30	1.001	0.990
22	94	92	30	0.993	0.995
25	107	104	30	0.981	0.995
30	128	123	30	0.965	0.995
35	146	140	30	0.952	0.995
40	163	154	30	0.942	1.000
45			30	0.934	1.000
50			30	0.930	1.000

Values from BEJGER et coll (1975) with cut-off energy $\Delta E = 15$ keV.

Values from JOHANSSON et coll (1977) for a cylindric ionization chamber with diameter 5 mm of tissue/water equivalent or air (graphite) equivalent material.

A plane parallel ionization chamber is recommended for $E_0 \leq 10$ MeV.

beams with E_0 above 10 MeV the absorbed dose at the reference point should be determined in a water filled polymethylmethacrylate or polystyrene phantom with outer dimensions at least $0.3 \text{ m} \times 0.3 \text{ m} \times 0.3 \text{ m}$. The thickness of the phantom walls oriented towards the radiation source should be 5 mm or less. The water filling should be at least 0.25 m. If the distance between the edge of the beam and the edge of the phantom becomes less than 50 mm at the en-

trance surface a larger water phantom should be used so that the distance will never be less than 5 mm. The ionization chamber should be protected during the water measurement by a tube with a wall thickness of 1 mm manufactured from polymethylmethacrylate. This tube should be attached to a holder that can be adjusted for measurement at various depths. The symmetry axis of the chamber must be positioned in the reference plane.

For electron energies in the range $1 \leq E_0 \leq 10$ MeV the absorbed dose in the reference point should be measured in a solid phantom. A plane parallel ionization chamber should be used (see supplement to be published).

The effective point of measurement for a cylindric ionization chamber is displaced from the centre of the chamber towards the radiation source (DUTREIX & DUTREIX 1966, HETTINGER et coll 1967). However for electron radiation the centre of the cylindric chamber may be placed at the reference depth as this is situated at a dose plateau or at least on a slow varying part of the depth ionization curve. For photon radiation the chamber centre shall be placed at the reference depth due to convenience. In this case correction factors are included in the total perturbation factors below in order to correct for displacement.

Measurements should be made for all combinations or irradiation conditions. A horizontal or vertical beam direction may be employed. With wedge fields the edge of the wedge should be placed parallel to the symmetry axis of the ionization chamber. The measurements should be made at the two possible 180° different orientations of the wedge and the average result should represent the absorbed dose.

The Bragg Gray equation is recommended for the determination of the absorbed dose at the reference point in the water in the absence of the chamber and the user's radiation quality $D_{w,u}$. Thus

$$D_{w,u} = D_{air,u} p \quad (s_{w,air})_u \quad (10)$$

where

- $D_{air,u}$ = mean absorbed dose to air in the cavity of the ionization chamber measured in water at the user's radiation quality in Gy
- $(s_{w,air})_u$ = mass stopping power ratio water to air at the reference point in the user's radiation quality
- p_u = total perturbation factor including corrections for lack of water equivalence in the

Table 6

Recommended values of $(s_w)_{\text{air}}$ and p for photon radiation at the reference point in a water phantom. The variations of the stopping power ratios with depth for depths beyond the absorbed dose maximum are considered to be negligible (NATHAN 1975)

Radiation beam quality	J/J_{ref}	$(s_w)_{\text{air}}^*$	p_{max}^*	p_{water}
Co- γ	1.97	1.150	0.970	0.990
4 MV	1.84	1.145	0.970	0.990
6	1.71	1.140	0.960	0.990
8	1.63	1.135	0.960	0.994
10	1.59	1.125	0.985	0.995
17	1.56	1.110	0.985	0.99
14	1.54	1.115	0.985	0.995
16	1.57	1.110	0.985	0.995
18	1.60	1.104	0.985	0.995
20	1.49	1.105	0.985	0.995
22	1.47	1.100	0.985	0.995
25	1.46	1.095	0.990	0.995
30	1.45	1.090	0.990	0.995
35	1.44	1.080	0.990	0.995
40	1.43	1.075	0.990	0.995
45	1.43	1.070	0.990	0.995
50	1.47	1.065	0.990	0.995

* Values from ICRU (1969)

■ values from JOHANSSON et coll (1977) for a cylindrical ionization chamber with diameter 5 mm

ionization chamber material at the user's radiation quality: perturbation of the fluence due to the insertion of the air cavity and location of the effective point of measurement of the cylindrical chamber due to the curved ionization chamber wall only for photon beams

With the assumption that

$$N_D = \frac{D_{\text{air}}}{M} \approx \frac{D_{\text{ref}}}{M}$$

the absorbed dose at the reference point in water is obtained from

$$D_w = N_D M_p (s_w)_{\text{air}} \quad (11)$$

where

M = meter reading at user's quality, corrected for temperature, pressure, recombination, humidity etc. in C or div

This is the essential equation for practical dosimetry work. p_k factors for different radiation beam qualities and ionization chamber materials are given in Tables 5 and 6. $(s_w)_{\text{air}}$ values are found in Table 7.

In the symbol $(s_w)_{\text{air}}$ is not specified the type of stopping power ratio. Two different sets of stopping power ratios should strictly be used: one for a chamber of air equivalent walls and another for a chamber of water equivalent walls. In the first case Harder's extended Bragg-Gray cavity theory (HARDER 1966) is to be used, which means that it is assumed that a delta ray equilibrium exists in the air cavity due to the air equivalent wall (i.e. the energy carried into the cavity by delta rays is balanced by energy carried out by delta rays). $(s_w)_{\text{air}}$ should then be the collisional mass stopping power ratio water to air. In the second case Spencer-Attix theory is to be used. The distribution of the electron fluence down to a certain cut-off energy must then be known at the point of measurement in the water phantom and the restricted stopping powers should be used for this distribution. The cut-off energy is dependent on the size of the chamber. However, in electron beams the two sets of data differ with less than one per cent when using the mean electron energy calculated according to BRAHME (1973) for determination of the collision stopping power ratios in the Bragg-Gray Harder theory and when using BERGER et coll (1975) electron fluence distribution to determine the mass stopping power ratio in Spencer-Attix theory. Furthermore, experiments in both electron and photon beams with air equivalent and water equivalent chambers show that the same stopping power data within about one per cent could be used for the two types of walls (JOHANSSON et coll 1977). Therefore for simplicity only one set of $(s_w)_{\text{air}}$ is recommended. The $(s_w)_{\text{air}}$ from BERGER et coll (1975) are recommended for electron radiation and those from the ICRU (1969, Table A.3) for photon radiation.

Illustrative example

Calibration at standards laboratory. The national standards laboratory has performed a calibration of the ionization chamber which has a diameter of 5 mm and a graphite wall of about 0.5 mm. A build-up cap of graphite was used at the calibration and the thickness of wall and cap together was 0.45 g cm⁻². The following information on the ionization chamber calibration factors are given in the calibration certificate:

The air kerma calibration factor N_K obtained for the ionization chamber in the ⁶⁰Co- γ ray beam at the laboratory at a field size 100 mm \times 100 mm and a focus-detector distance of 1 m was found to be

$$N_K = 1.10 \text{ Gy/div at } 22^\circ \pm 0.1^\circ, 101.33 \text{ kPa and } 50\% \text{ rel. air humidity}$$

Table 7

Recommended values of $(s_w, 1)$ as a function of depth (z) and mean energy at the phantom surface \bar{E} for electron radiation. The values are taken from BERGER et al. (1975) with energy cut off $\Delta=15$ keV, $(h_{att})=71.3$ eV and $(h_{air})=97.9$ eV.

Depth ($s_w, 1$) mm	Mean energy at phantom surface \bar{E} (MeV)							
	1	2	3	4	5	6	7	8
1	1.136	1.112	1.097	1.074	1.058	1.043	1.031	1.019
2	1.144	1.124	1.101	1.081	1.065	1.049	1.037	1.025
4	1.151	1.137	1.112	1.090	1.072	1.056	1.042	1.031
6	1.157	1.147	1.122	1.099	1.080	1.063	1.048	1.037
8		1.154	1.132	1.108	1.088	1.070	1.055	1.042
10		1.157	1.142	1.118	1.097	1.078	1.061	1.048
12			1.150	1.129	1.106	1.086	1.068	1.055
14			1.155	1.139	1.115	1.094	1.074	1.061
16			1.157	1.146	1.125	1.104	1.082	1.068
18				1.151	1.134	1.112	1.091	1.074
20				1.155	1.141	1.121	1.100	1.082
25				1.156	1.153	1.141	1.120	1.102
30					1.154	1.151	1.137	1.122
35						1.152	1.148	1.139
40								1.147
45								1.146

Depth ($s_w, 1$) mm	Mean energy at phantom surface \bar{E} (MeV)							
	9	10	12	14	16	18	20	22
0	1.011	1.002	0.990	0.980	0.972	0.965	0.958	0.952
5	1.073	1.015	1.001	0.990	0.981	0.974	0.969	0.962
10	1.036	1.027	1.012	1.000	0.990	0.982	0.975	0.968
15	1.050	1.038	1.023	1.009	0.998	0.989	0.982	0.974
20	1.066	1.053	1.033	1.018	1.006	0.997	0.988	0.980
25	1.081	1.069	1.045	1.027	1.013	1.004	0.995	0.986
30	1.102	1.087	1.058	1.037	1.022	1.011	1.001	0.993
35	1.110	1.105	1.072	1.049	1.031	1.018	1.008	0.999
40	1.138	1.122	1.086	1.060	1.041	1.025	1.015	1.005
45	1.144	1.137	1.104	1.075	1.053	1.034	1.022	1.011
50		1.144	1.120	1.091	1.065	1.046	1.031	1.017
60			1.138	1.119	1.093	1.069	1.051	1.035
70				1.135	1.117	1.093	1.072	1.055
80					1.127	1.114	1.094	1.073
90						1.122	1.118	1.097
100							1.119	1.118
110								1.114
120								1.105

Depth ($s_w, 1$) mm	Mean energy at phantom surface \bar{E} (MeV)					
	25	30	35	40	45	50
0	0.944	0.931	0.923	0.917	0.912	0.907
10	0.959	0.945	0.935	0.926	0.919	0.914
20	0.971	0.956	0.944	0.934	0.928	0.924
30	0.981	0.965	0.952	0.942	0.934	0.929
40	0.992	0.974	0.960	0.949	0.940	0.935
50	1.003	0.983	0.968	0.956	0.946	0.941
60	1.016	0.993	0.977	0.963	0.951	0.946
70	1.030	1.004	0.986	0.971	0.960	0.955
80	1.047	1.016	0.995	0.979	0.967	0.962
90	1.065	1.030	1.006	0.988	0.974	0.969
100	1.085	1.045	1.017	0.997	0.982	0.977
110	1.112	1.062	1.030	1.006	0.991	0.986
120	1.122	1.079	1.043	1.017	0.999	0.994
130	1.117	1.101	1.058	1.028	1.008	1.003
140	1.105	1.113	1.073	1.041	1.018	1.013
160	1.099	1.105	1.103	1.068	1.040	1.035
180		1.094	1.101	1.094	1.064	1.059
200			1.094	1.094	1.088	1.083
220				1.081	1.088	1.083
240				1.083	1.075	1.070
260					1.072	1.067
280						1.067

The absorbed dose to air (inside the cavity) ionization chamber factor N_D recommended in the present protocol is

$$N_D = N_A k_{att} k_m (1-g)$$

$$N_A = 1.10 \text{ Gy/div}$$

$$k_{att} = 0.990$$

$$k_m = 0.991$$

$$g = 0.004$$

$$N_D = 1.075 \text{ Gy/div}$$

$$\text{at } 22^\circ \text{C } 101.33 \text{ kPa and } 40\% \text{ rel air humidity}$$

Measurements in an electron beam (1) A depth ionization curve is measured for a large beam size at SSD=1.0 m in order to determine the mean energy of the electrons at the phantom surface E_0 . In the measurements the effective point of measurement (p_{71}) is used. The depth at which the ionizations are reduced to 50 per cent (R_{50}) is determined to 84 mm. From Fig. 3 the corresponding $L_{0.5}$ value is obtained and $L_0=20$ MeV.

(2) From the depth ionization curve a depth absorbed dose curve is calculated using $(s_w, 1)$ from Table 7. The depth of the maximum absorbed dose w is found to be 30 mm. The absorbed dose

this reference depth is then determined from a measurement with the centre of the ionization chamber at this depth. Insertion of the meter reading M (corrected for temperature, pressure, recombination etc.) and p and $(s_{w,air})_u$ factors from Table 5 in eq. (11) gives the absorbed dose in water at the reference point

$$\left. \begin{aligned} D_{w,u} &= N_D M p (s_{w,air})_u \\ N_D &= 1.075 \text{ Gy/div} \\ p &= 0.990 \\ (s_{w,air})_u &= 1.001 \end{aligned} \right\} D_w = 1.065 M_0 \text{ Gy}$$

3. *Measurements in a photon beam* (1) The photon beam quality was estimated from the ratio J_{100}/J_∞ which was 1.51. This corresponds roughly to a maximum photon energy of 17 MeV according to Table 6 or Fig. 4. The reference depth is then obtained from Table 3 as 100 mm. The absorbed dose at this depth is measured with the centre of the ionization chamber at this point. If M_u is the meter reading (corrected for temperature, pressure, recombination etc.) eq. (11) and Table 6 give

$$\left. \begin{aligned} D_{w,u} &= N_D M_u p_u (s_{w,air})_u \\ N_D &= 1.075 \text{ Gy} \\ p &= 0.985 \\ (s_{w,air})_u &= 1.108 \end{aligned} \right\} D_w = 1.173 M \text{ Gy}$$

(2) The absorbed dose at dose maximum is determined from the ratio of the depth ionization at 100 mm and dose maximum. The depth ionization curve is used for evaluation of this ratio. (The effective point of measurement $0.75r$ should be used in measuring depth ionization curves.)

Determination of absorbed dose at any point

Relative absorbed dose distributions should be related in the absorbed dose at the reference point which should therefore be included in all distribution determinations. The distribution should apply to a large water phantom (page 68). A complete set of distributions should be available for all combinations of energy, field sizes, SSD etc. that are in use for radiation therapy. The hospital physicist is responsible for all modifications of these distributions in clinical practice, for instance the insertion of lead block and bolus.

Beam axis absorbed dose distribution

Electron beams The beam axis depth absorbed dose distribution cannot be specified in a unique

way from electron beam parameters such as energy, field size and SSD. The shape of the distributions is dependent on a large number of constructional details of the accelerators and they are only partly contained in the beam parameters. Therefore as a rule the distributions should be determined for each accelerator. When accelerators of the same design are used (SVENSSON 1971) common beam axis depth dose distributions may be applied by departments only after a check of some distributions by measurements. Significant differences may appear as a result of individual adjustments and small differences in thickness of accelerator window foils and in collimator design etc.

The relative depth absorbed dose distributions could be measured with ionization chambers, semiconductor detectors, liquid ionization chambers or ferrous sulphate dosimeters. The choice of method depends on the instruments that are locally available. The relative distributions should be checked against depth absorbed dose curves measured with an ionization chamber method or possibly when available ferrous sulphate dosimeters.

The ionization chamber method is well established for measurements at depths equal to or larger than that of the dose maximum. In the measurements of relative depth ionization curves the displacement effect of the ionization chamber should be taken into account for cylindrical chambers. This effect should be corrected for by using an effective point of measurement. This point has been determined by extrapolating the geometric displacement of the depth ionization curves measured by different sizes of cylindrical chambers to a zero size chamber. In the experimental determination the perturbation effect was not considered, so this is automatically corrected for in the use of the effective point of measurement. The effective point of measurement varies slightly with the electron energy $E_{0,e}$ and phantom depth and is $0.5r$ to $0.75r$ in front of the chamber centre where r is the radius (HETTINGER et coll. 1967; DUTREIX & DUTREIX 1966; JOHANSSON et coll. 1977). A value of $0.5r$ is recommended for electron radiation. The recombination losses can be disregarded in the measurement of relative depth ionization curves for those chambers recommended in the present protocol for most treatment units. The relative depth ionization curves should be multiplied with $(s_{w,air})_u$ (Table 7) for different depths in order to convert these curves to relative depth absorbed dose curves.

At small phantom depths the air ionization method might introduce uncertainties due to contamination of low energy electrons and an incomplete build up of δ ray spectrum. If measurements at such depths are desired a liquid ionization chamber (HULTÉN & SVEINSSON 1975) or thin ferrous sulphate dosimeters (SVEINSSON & HETTINGER 1967) could be recommended. Since in general it is only necessary to make these measurements in the calibration procedure after an accelerator installation joint measurements by different departments are recommended.

It is often useful to investigate the therapeutic and physical properties of an electron beam and describe them by some simple parameter. There are several reasons for introducing such parameters (BRAHME & SVEINSSON 1976) thus the increasing number of electron accelerators of different types and often quite different beam qualities necessitates a simple unified comparison of the quality of one beam with another. The desirability of simple and accurate beam diagnostics make it useful to focus attention on a few independent parameters characterizing the beam quality and finally from a therapeutic point of view it is important to define the treatment volume in a relevant and consistent way not least to simplify comparisons between different treatment centres.

The parameters that are recommended for use appear in Fig. 2. D_m the maximum absorbed dose along the beam axis, D the surface absorbed dose at 0.5 mm depth (this depth has been chosen as it is accessible for accurate absorbed dose measurement and as it approximately corresponds to the radiation sensitive layer below the epidermis), D_p the photon background, G the absorbed dose gradient, R_{100} the depth of the absorbed dose maximum, R_{85} the therapeutic range, R_{50} the half value depth and R_p the practical range.

Of particular importance for an electron beam are R_{85} and G . Experimental and theoretic values for broad beams are given in Figs 6 and 7. A dose gradient below about 2.5 for large beams indicates that the scattering system and collimating system are of poor design and that unnecessary large volumes of normal tissue are irradiated in single beam technique.

Photon beams The beam axis depth absorbed dose curves are less critically dependent on the beam parameters and simpler to measure than for electron radiation. All the dosimeter systems men-

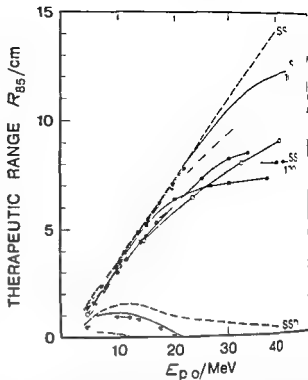


Fig. 6 The therapeutic range R_{85} for large field sizes as a function of the most probable energy at the phantom surface E_p . The SSD=1 m if not otherwise stated. Experimental points are indicated with different symbols for different accelerators. The upper two curves are theoretic data from BERGER & SELTZER (1969); the SSD=1 m curve is derived from inverse square law corrections. Near surface lines are obtained for those beams with $D/D_m < 0.85$.

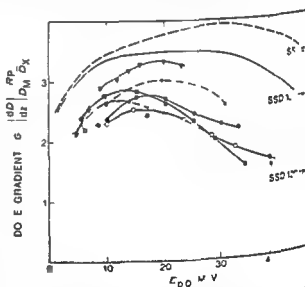


Fig. 7 Dose gradients G for large field sizes as a function of the most probable energy at the phantom surface E_p . The SSD=1 m if not otherwise stated. Experimental points are shown with different symbols for different accelerators. The upper curves are theoretic data from BERGER & SELTZER (1969); the SSD=1 m curve is derived from inverse square law corrections.

tioned for electrons can be used but should be checked against the air ionization method. The difference between the relative depth absorbed

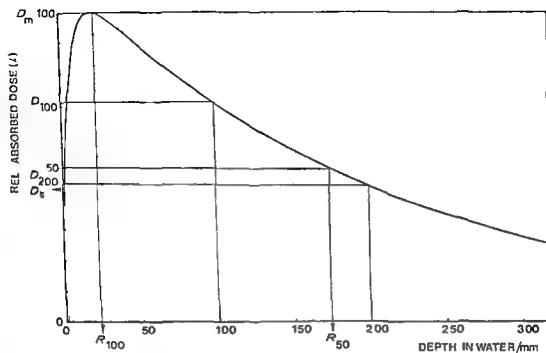


Fig 8 Beam axis depth absorbed dose distribution for photon beams. Parameters often used to characterize the quality of the

distribution are indicated $D_{0.01}/D_{0.05} \approx J_{0.01}/J_{0.05}$ which is input parameter for (s_w) in Table 6

and depth ionization curves when both are normalized at maximum may be 1 to 2 per cent at a large depth (SVENSSON 1971 NAHUM 1975). These differences can be disregarded for practical dosimetry. In the measurement of depth ionization the displacement effect must be considered and the effective point of measurement must be used. The effective point of measurement varies slightly with energy and phantom depth (HETTINGER et coll 1967 JOHANSSON et coll 1977). A value of 0.75 r is recommended for all energies and phantom depths. The variations can be disregarded in practical dosimetry. Published depth dose data for different peak energies are available for different accelerators. Those data should be checked by measurements of a few distributions both at small and large field sizes.

Parameters describing the physical and therapeutic properties of the beam axis absorbed dose distributions are also useful for photon radiation. D_1 , D_m , R_{100} , R_{50} are defined as for electron radiation (Fig 8). The ratio J_{100}/J_{200} is discussed on page 62 and is of importance for the choice of stopping power ratios.

Iso-absorbed dose distributions

A semi-conductor detector connected to an automatic recorder may be used for the absorbed dose distribution determination in a water phantom for

both photon and electron radiation beams. This method is simple as the isodoses are directly plotted and corrections are often not necessary to carry out. The spatial resolution is good as the sensitive layer of the detector is less than a few mm². Systematic errors could however be introduced for some accelerators especially for betatrons dependent on the radiation pulse shape and neutron contamination. Furthermore the curves measured with the semiconductor may differ from the relative isodose curves at small phantom depths (BRAHME & SVENSSON 1976). The method should therefore be checked against ionization chamber measurements or possibly when available ferrous sulphate dose meters.

The absorbed dose of electron beams at points outside the beam axis may be assessed by means of photographic film placed parallel to the beam axis in polystyrene phantoms. A significant difference between the relative depth absorbed dose curve and the relative depth blackening curve exists at depths smaller than 20 mm (LOEVINGER et coll 1961 HETTINGER & SVENSSON 1967). The beam axis depth absorbed dose curve is first measured and is then used to assign a depth absorbed dose value to all points along the beam axis in the film. Isodensity curves joining points in the film with the same net blackening are assigned to the depth absorbed dose

Table 8
Suggestion of a maintenance program for accelerators and ^{60}Co γ units

Check of		Frequency of check				
		Once a day	Once a week	Once a month	Once a quarter	Once a year
Light beam and pointers	p 74	^{60}Co acc				
Radiation beam and light beam agreement	p 74 Fig 5		^{60}Co acc			
All mechanical alignments	p 74 HPA (1970) AAPM (1975)					^{60}Co acc
Abs dose monitor and patient dose agreement	p 75	^{60}Co acc				
Abs dose monitor calibr factor constancy	p 75		acc	Co		
Abs dose monitor calibr factor (independence of diff param	p 75			acc	acc	
Energy constancy	p 75		acc			
Radiation beam uniformity	p 75		acc			^{60}Co

at the point where they pass the beam axis. It is frequently sufficient to construct the isodensity curves without correcting for background blackening.

The same method for photon beams as for electron beams may be employed although the accuracy of this method in the penumbra region is less satisfactory. The film method is often less accurate than measurements with semiconductor detectors. Another approach is to use transversal measurements at 4 depths with an ionization chamber (decrement line method ORCHARD 1964, ORR et al 1964) followed by computer calculation of the isodose curves (KALNIA & MUNK 1972).

Maintenance program for the dosimetry of therapy units

The dosimetry data on which the irradiations are based shall be checked regularly for constancy. The number of checks may depend to some extent on the behaviour of the particular therapy unit and its intended use. If previous examinations indicate few and slow changes some decrease in their frequency may be satisfactory. A maintenance program for ^{60}Co γ units and accelerators is suggested in Table 8 where the frequencies are given on the assumption that dual absorbed dose monitoring (page 75) exists and that measurements of absorbed doses on patients are performed (page 76). All technical

checking procedures prescribed by the manufacturer should be followed.

The responsible physicist should for each therapy unit write instructions on how to carry out the relevant checks and their frequencies. A logbook for recording these measurements shall be kept.

Radiation beam alignment checks

A simple check on the light beam should be carried out daily. A white card on which is drawn a square field is placed at the normal SSD. With one of the numerical field size indicators a corresponding field size is set up and the light field is compared with the drawing. Without moving the card this comparison is again performed after the radiation head is rotated through 180° . It shall be checked that the cross hair light image and the front pointer indicate the centre of the light field and that the cross hair is projected on the back pointer tip.

Checks of agreement between the light beam and the radiation beam should be performed every week and each time the light beam bulb is exchanged. It is often convenient to combine this check with that of the radiation beam uniformity. The agreement should fulfil the values given in Geometric considerations (page 10).

Once a year a thorough alignment test shall be performed. Detailed information on relevant procedures has been given (HPA 1970, AAPM 1976).

Absorbed dose monitor checks

Whenever performed the measurements of absorbed doses on patients should be checked for agreement with the absorbed dose monitor.

On accelerators the constancy of the absorbed dose monitor calibration should be checked weekly. It should be possible to relate this check to the primary absorbed dose calibration of the monitor. A special phantom made of plastic should be used because of the convenience of handling. The position of the dosimeter should be close to the reference depth (Table 3). As several different reference depths might be of interest the phantom may consist of a basic block together with a series of slabs marked and used for this purpose only. Monthly to quarterly a suitable series of such measurements could be performed for testing the monitor precision and stability and for examination of the calibration factors for independence on monitor setting, absorbed dose rate, beam direction, temperature and air pressure. Also the dependence of the calibration factor on wedges and field size, flattening filter or scattering foils should be checked for constancy.

On ^{60}Co γ units the check of the absorbed dose monitor calibration may be less frequent but at least once a month. The check reveals timer errors, changes in shutter effects and possible changes in absorbed dose rate caused by, for instance, redistribution of the source (HANSEN 1972).

The ratio between the monitor reading and the determined absorbed dose value shall not deviate by more than ± 2 per cent from the ratio determined at the original measurements.

Radiation energy constancy checks

On therapy units which could have an unintentional change in the selected radiation energy (i.e. ^{60}Co linear accelerators) the energy constancy should be checked once a week. This is performed by checking the constancy of a ratio J_1/J_2 between ionization measurements at two different depths. The phantom for check measurements of absorbed dose monitor calibration should be used and J_1 is the measurement at the reference depth. J_2 is measured with an additional slab plastic material in front of the phantom. For photon beams this slab should be approximately 10 cm thick and for electron beams about R_{50} minus the reference depth (Fig. 2, Table 3). The best geometric reproducibility is obtained if the source-chamber distance is unaltered

between measurements. In some irradiation geometries this is not possible and the SSD should instead be kept constant. The ratios J_1/J_2 should be related to the relevant energy calibrations and the plastic slabs should be marked and used for this purpose only.

The ratios J_1/J_2 should not deviate by more than ± 1 per cent from the original ratios for photon beams. For electron beams the deviation should be less than ± 4 per cent.

Radiation beam uniformity checks

The uniformity should be checked with the photographic film method (page 64). In each check the maximum blackening of the film and the blackening along the major axes and diagonals of the field should at least be determined. A full evaluation of the film blackening is of great value as the uniformity index then may be determined. The check should be made weekly with cyclic permutation of some relevant irradiation conditions (e.g. radiation quality, beam direction and field size). i.e. each combination is checked at least every month. The minimum requirements given in the section Geometric considerations should be fulfilled in all uniformity measurements.

Checks of absorbed dose given to the patient

The absorbed dose given to each patient shall be under proper control (ICRP 1970, LINDELL 1976, ICRU 1976). For this purpose dual absorbed dose monitoring systems and measurements of the absorbed dose on patients are recommended. The dual monitoring system shall protect the patient against overdoses caused by equipment failures. The patient dose measurements should detect any erroneous absorbed dose caused by equipment malfunctions and human mistakes so that proper corrections in the following treatments can be performed.

Absorbed dose monitoring systems

Malfunctioning of ^{60}Co - γ unit timers have been reported (VELKLEY 1975). Also mechanical malfunctions in the beam control system (sticking shutter, broken return spring, etc.) have caused excessive and unknown absorbed doses to patients. It is recommended to provide ^{60}Co - γ units with two independent timer systems both capable to give a termination signal to the beam control system. Detailed recommendations are given in Radiotherapy Ap-

paratus Safety Medical Panel (RASMP 1975). Dual timer systems coping with these recommendations are commercially available.

Accelerators shall be provided with two independent absorbed dose monitoring systems which to advantage should have physically separated radiation detectors. At least one detector system shall consist of a transmission chamber and measure the full beam at the patient side of any flattening filter or scattering foils. Both monitors shall be capable of independently terminating the irradiation. The integrated signals from the detectors should be digitally displayed on the console and at least one monitor should start from zero at the beginning of an irradiation. The readings of at least one display instrument should be preserved in the event of any failure (including power failure) or interruption of the irradiation. It should not be possible to start another treatment before the dose monitors and the presettings are reset. The absorbed dose rate shall be indicated on the console and for each increment of the dose monitor readings an acoustic beat should be given. If the dose rate exceeds a certain preset level (e.g. twice the normal dose rate) automatic termination of the irradiation should result. From the radiation detectors signals should be derived which are proportional to the output in different parts of the radiation beam so that the beam uniformity can be detected. An abnormal uniformity should result in an automatic termination of the irradiation. For a given radiation quality the dependence of the dose monitor calibration factor on dose rate, beam direction, temperature and air pressure should be within 2 per cent. The dependence on beam energy, field size and flattening filter or scattering foil should be less than ± 30 per cent.

Absorbed dose measurements on patients

Absorbed dose measurements on patients could be divided into two levels of security. For level one measurements are made at one of the first treatment occasions and every time a parameter (including patient anatomy) is changed. This enables detection of systematic mistakes in the decided irradiation procedure. Level two measurements are made at every treatment occasion enabling detection of occasional operator mistakes and equipment failures.

Preferably level one measurements should be carried out in more than one point of the field. A further improvement may be obtained in the reduction

of the number of occasional operator mistakes. Level one measurements are correlated to a set and confirm system. Occasional changes in positioning and occasional equipment failures can, however, be revealed only by level two dosimetry. On older radiation units with only one dose measurement system level two measurements may also replace a secondary dose monitor. A thorough discussion of possible errors and control of the absorbed dose to the patient for specific therapy procedures is given by MÖLLER et al. (1976).

Patient dosimetry systems to be used routinely should besides reasonable precision possess simplicity of handling. The demand for precision is determined by an action level for open beams of ± 1 per cent. For beams modified by a wedge or compensating or blocking filter an action level of ± 4 per cent should be used. At least small condenser ionization chambers (Sievert chambers), the luminescent dosimeters (TLD) and small semiconductor detectors with suitable (partial) built-in caps can fulfil these requirements.

Appendix

For a proper understanding of the protocol definitions are necessary taken from ICRU (1973), HPA (1970) and NACP (1972) or slightly modified.

Beam. The electron or photon beam is the region in space traversed by photons or electrons from source. Its edges are determined by the collimator, its cross sections perpendicular to the beam axis, the field and its direction is that of photon or electron travel.

Beam axis. Four types of beam axes can be defined. In a properly adjusted system all four will coincide. The definitions are:

(1) Mechanical definition. The *collimator axis* is defined as the rotation axis at the collimator head.

(2) Geometric definition. The *geometric beam axis* is defined as the line passing through the centre of the beam flattening filter (or the main scattering foil or focus of scanning magnet system) and the centre of the final beam limiting diaphragm.

(3) Radiation definition. The *radiation beam axis* (sometimes named reference axis) is defined as the line passing through the centre of the effective radiation source and the centre of gravity of the region within which the absorbed dose exceeds 50 per cent of the maximum absorbed dose at the reference plane in a phantom.

(4) Light beam definition the *light beam axis* is defined as the line from the effective light source and the centre of gravity of the area within which the light intensity exceeds 50 per cent of the maximum light intensity at the phantom surface

Reference plane The reference plane is defined as the orthogonal plane to the reference axis at a given depth beneath and parallel to the phantom surface. Recommended values for the depth are given in Table 3

Reference point The reference point is defined as the point of the intersection between the reference plane and the beam axis (reference axis)

Radiation field size The field size is defined in phantom at the depth of the reference plane with the reference plane at the proposed treatment distance. The field size is the area inside the 50 per cent level of absorbed dose in the reference point. The numerical values of the field size are given as the distance between the 50 per cent level at the edges of the major axes or the diameter of the 50 per cent level in a circular field

Light field size The light field size is defined at the surface of a phantom with the surface at the

proposed treatment distance. The light field size is the area inside the 50 per cent boundary of the light intensity, the light intensity in the centre being 100 per cent. The numerical values of the field size are given as the distance between the boundary of the major axes

Uniformity index The uniformity index is defined in the reference plane for a specified quantity (e.g. absorbed dose, ionization, net film density or current from a semiconductor detector) as the ratio of the area containing points where this quantity exceeds 90 per cent of its value at the reference point and the area where it exceeds 50 per cent of the reference point value

Physical penumbra Physical penumbra is for a specified quantity as the lateral distance at the major axes between the 80 per cent and the 20 per cent of points of this quantity with the value at the reference point defined as 100 per cent

Reprints may be obtained from L. Lindborg, National Institute of Radiation Protection, Box 60204, S 104 01 Stockholm, Sweden

REFERENCES

- LEMOND P R and SVENSSON H Ionization chamber dosimetry for photon and electron beams. *Acta radiol Ther Phys Biol* 16 (1977) 177
- AMERICAN ASSOCIATION OF PHYSICISTS IN MEDICINE (AAPM) Code of practice for X ray therapy linear accelerators. *Med Phys* 2 (1975) 110
- ROGER M J and SELTZER S M Quality of radiation in a water medium irradiated with high energy electron beams. Paper presented at the 17th International Congress of Radiology, Tokyo, Japan, October 1969
- , DOMEN S R and LAMPERTI P J Stopping power ratios for electron dosimetry with ionization chambers. In *Biomedical dosimetry* (proceedings of a symposium, Vienna 1975) IAEA 1975
- OUTILLOU M Some remarks concerning the measurement of kerma with a cavity ionization chamber. *Bulletin International des Poids et Mesures* Nov 1977 (CCEMRI (1)/77 114)
- OAG J W Ionization chambers. In *Radiation dosimetry* Volume II, p 1. Edited by F H Attix and W C Roesch. Academic Press Inc, New York, 1966
- RAHNE A Investigations on the application of a Microtron accelerator for radiation therapy. Theis University of Stockholm, 1975
- and SVENSSON H Specification of electron beam quality from the central axis depth absorbed dose distribution. *Med Phys* 3 (1976) 95
- Radiation beam characteristics of a 22 MeV Microtron. *Acta radiol Oncology* 18 (1979) 244
- DUTREIX A Film dosimetry. In *American Association of Physicists in Medicine Summer school* University of Vermont, 1976
- DUTREIX J et DUTREIX A Etude comparée d'une série de chambres d'ionisation dans les faisceaux de 20 et 10 MeV. *Biophysik* 3 (1966) 249
- GERMAN STANDARD ASSOCIATION DIN 6809/1 Clinical dosimetry, therapeutic application of X ray, gamma ray and electron beams, 1976
- (a) DIN 6800/1 Procedures in dosimetry, principles of photon and electron dosimetry with probe type detectors, 1975 (Draft)
- (b) DIN 6800/2 Procedure in dosimetry, ionization dosimetry, 1975 (Draft)
- HANSEN H Apparent rapid decay of a ^{60}Co teletherapy source (abstract). In *Proceedings of the sixth conference of the Nordic Association of Clinical Physicists* Edited by C H Madsen and K Lidén. *Acta radiol* (1972) Suppl No 313 p 287
- HARDER D Physikalische Grundlagen der Dosimetrie Strahlentherapie. Suppl. Deutscher Röntgenkongress 1965
- and SCHULZ H J Some new physical data for electron beam dosimetry. In *Proceedings of the European*

- Congress of Radiology Amsterdam 1971 Excerpta Medica Amsterdam 1972
- HETTINGER G and SVENSSON H Photographic film for determination of isodose curves from betatron electron radiation Acta radiol Ther Phys Biol 5 (1967) 74
- PETTERSSON C and SVENSSON H Displacement effect of thimble chambers exposed to a photon or electron beam from a betatron Acta radiol Ther Phys Biol 6 (1967) 61
- HOSPITAL PHYSICISTS' ASSOCIATION (HPA) A code of practice for the dosimetry of 2 to 35 MV X ray and Caesium 137 and Cobalt 60 gamma ray beams Phys in Med Biol 13 (1969) 1
- A suggested procedure for the mechanical alignment of telegamma and megavoltage X ray beam units HPA Report Series No 3 1970
- A practical guide to electron dosimetry 5–35 MeV HPA Report Series No 4 1971
- A practical guide to electron dosimetry below 5 MeV for radiotherapy purposes HPA Report Series No 13 1975
- HULTÉN G and SVENSSON H Electron depth absorbed doses for small phantom depths Acta radiol Ther Phys Biol 14 (1975) 537
- INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION (ICRP) Publication 15 Protection against ionizing radiation from external sources Pergamon Press Oxford 1970
- INTERNATIONAL COMMISSION ON RADIATION UNITS AND MEASUREMENTS (ICRU) Report No 10b Physical aspects of irradiation NBS Handbook 85 Washington DC 1964
- Report No 14 Radiation dosimetry X rays and gamma rays with maximum photon energies between 0.6 and 50 MeV Washington DC 1969
- Report No 19 Radiation quantities and units Washington DC 1971
- Report No 21 Radiation dosimetry Electrons with initial energies between 1 and 50 MeV Washington DC 1972
- Report No 24 Determination of absorbed dose in a patient irradiated by beams of X or gamma rays in radiotherapy procedures Washington DC 1976
- Report No 26 Neutron dosimetry for biology and medicine Washington DC 1977
- Report No 31 Average energy required to produce an ion pair Washington DC 1979
- JOHANSSON K A BENGTSOHN E and LINDSKOUG B A digital electrometer In Proceedings of the sixth conference of the Nordic Association of Clinical Physics Edited by C B Madsen and K Lidén Acta radiol (1972) Suppl No 313 p 76
- MATSSON L O LINDBORG L and SVENSSON H Absorbed dose determination with ionization chambers in electron and photon beams with energies between 1 and 50 MeV In International symposium on national and international standardization of radiation dosimetry Atlanta 1977 (IAEA SM 222/35)
- KALNAY O and MUNK J Automatic production of isodose curves Acta radiol Ther Phys Biol 11 (1972) 90
- LINDELL B (Editor) Report on the applicability of international radiation protection recommendations to the Nordic countries Published by the radiation protection institutes in Denmark Finland Iceland Norway and Sweden Liber Tryck Stockholm 1976
- LOEVINGER R KARMARK C J and WEISSELTZ Radiation therapy with high energy electrons Physical considerations 10 to 60 MeV Radkly (1961) 906
- MARKUS B Energiebestimmung schneller Elektronen Tiefendosiskurven Strahlentherapie 116 (1961) 1
- Beiträge zur Entwicklung der Dosimetrie mit Elektronen Strahlentherapie 124 (1964) 33
- MAUDERLY W and BRUNO F P Solid state electron amplifier Phys in Med Biol 11 (1966) 43
- MOLLER F R NORDBERG U B GLSTAFSSON JOHANSSON J E LANDBERG T G and SVANEN T G Planning control and documentation of electron beam therapy Acta radiol (1976) Suppl No 341
- NAHUM A E Calculations of electron flux spectra in water irradiated with megavoltage electron and photon beams with applications to dosimetry Thesis University of Edinburgh 1975
- Water/air mass stopping power ratios for megavoltage photon and electron beams Phys in Med Biol (1978) 24
- NAYLOR G P and CHIVERALLS K The stability of an X ray beam from an 8 MV linear accelerator for radiotherapy Brit J Radiol 43 (1970) 414
- NORDIC ASSOCIATION OF CLINICAL PHYSICS (NAC) Procedures in radiation therapy dosimetry up to 50 MeV electrons and roentgen and gamma rays with maximum photon energies between 1 and 50 MeV Acta radiol Ther Phys Biol 11 (1972) 603
- ORCHARD P G Decrement lines A new presentation of data in Cobalt 60 beam dosimetry Brit J Radiol (1964) 756
- ORR J S LAURIE J and WAKERLEY S A study of MeV transverse data and associated methods of constructing isodose curves Phys in Med Biol 9 (1964) 505
- PODGORSAK E B RAWLINSON J A and JOHNS H X ray depth doses from linear accelerators in the energy range from 10 to 32 MeV Amer J Roentgenol 123 (1975) 182
- RADIOTHERAPY APPARATUS SAFETY MEDICAL PHYSICS (RASMP) Requirements for dose control of high energy X ray and electrons United Kingdom Department of Health and Social Security Document 1975
- RASSOW J IRDMANN U und STRUTER H D Bestimmung der Filmdosimetrie energiereicher Strahlung Strahlentherapie 138 (1969) 149
- SFITZER S M HUBBELI J H and BERCTER M J Theoretical aspects of electron and photon dosimetry In International symposium on national and international standardization of radiation dosimetry Atlanta 1977 (IAEA SM 222/05)
- SUB-COMMITTEE ON RADIATION DOSIMETRY OF THE AMERICAN ASSOCIATION OF PHYSICISTS IN MEDICINE

- (SCRAD) Protocol for the dosimetry of high energy electrons Phys in Med Biol 11 (1966) 505
- Protocol for the dosimetry of X and gamma ray beams with maximum energies between 0.6 and 50 MeV Phys in Med Biol 16 (1971) 379
- VENSSON H Dosimetric measurements at the Nordic medical accelerators II Absorbed dose measurements Acta radiol Ther Phys Biol 10 (1971) 631
- and HETTINGER G Measurement of doses from high energy electron beams at small phantom depths Acta radiol Ther Phys Biol 8 (1967) 289
- — Dosimetric measurements III the Nordic medical accelerators I Characteristics of the radiation beam Acta radiol Ther Phys Biol 10 (1971) 369
- VELKLEY D E * Co therapy machine malfunctions Med Phys 2 (1975) 125

MITOMYCIN C IN ADVANCED GALLBLADDER CARCINOMA

F VON EYBEN C HELLEKANT W MATYSSON U LJUNGQUIST
and K JONSSON

Carcinoma of the gallbladder has an overall 5 year survival of about 5 per cent (WARREN et coll 1968 EVIN et coll 1976 PERPETUO et coll 1978). Prognosis is correlated mainly with the stage of invasion (EVIN et coll) and to some extent with the sex and initial performance status (PERPETUO et coll). Tumor growth in the mucosa only (stage I) or in the mucosa and muscularis (stage II) had a 5 year survival of about 65 per cent (NEVIN et coll). Tumor spread through the wall of the gallbladder to the serosa (stage III) or to the regional lymph nodes (stage IV) had a 5 year survival of about 6 per cent (EVIN et coll). Patients with tumor spread beyond the regional lymph nodes (stage V) all died within one year. Gallbladder carcinoma treated with intravenous or intraarterial mitomycin C has shown objective remission (SHIRAHARA 1968 GUERRO et coll 1972 MOERTEL 1973 SHIGENAGA 1976). Patients with advanced gallbladder carcinoma have been treated with intravenous and intraarterial infusions of mitomycin C at this hospital and the results are now reported.

Material and Methods

Between 1977 and 1979 10 patients (8 women and 2 men) with microscopically confirmed advanced gallbladder carcinoma were treated. Median age was 72 years (range 48-78) at the time of diagnosis. Six patients had performance status I, two patients had performance status II, and two patients had performance status III (ZUBROD et coll 1960). Two patients had gallbladder carcinoma stage III and 8

stage V. Cholecystectomy had been performed in 7 patients, explorative laparotomy in 3, ileocecal resection in one, regional lymph node extirpation in one, and choledochotomy in one. One patient was treated with melphalan and medroxyprogesterone acetate before mitomycin C. None of the patients had been irradiated. All patients had a measurable tumor, a leukocyte count above 4×10^9 cells/l and a platelet count above 125×10^9 /l at the beginning of the mitomycin C treatment (Table 1).

Before each course, Hb, white blood cell count and platelet count were determined in all patients and the patients were examined physically. Hepatic and renal function tests were carried out regularly as well as scintigraphy of the liver and ultrasound examinations repeatedly to evaluate the response. The coeliac or the hepatic arteries were catheterized for the intraarterial infusions (Fig. 1). Seven patients were given 2 to 5 courses of mitomycin C intraarterially with 10 to 24 days interval. For infusion 10 mg mitomycin C was dissolved in 100 ml isotonic sodium chloride solution or 15 mg mitomycin C was dissolved in 150 ml isotonic sodium chloride solution. The infusion was given at a rate of 6 ml/min. After each infusion the catheter was withdrawn and the procedure was repeated for each intraarterial infusion. After the intraarterial treatment 2 patients were given 5 fluorouracil 1000 mg/m² body surface intravenously in 6 hours on day 1 and day 2 and

From the Departments of Oncology and Radiation Therapy, Diagnostic Radiology, Surgery, and Obstetrics and Gynecology, Malmö Allmänna Sjukhus S-205 21 Malmö, Sweden.
Submitted for publication 9 November 1979.



Fig. 1. Hypervascular carcinoma of the gallbladder. Case 10.

mitomycin C 6 mg/m² intravenously in 30 min on days 2 with 3 weeks' intervals. Two patients received a single intravenous infusion of mitomycin C.

All other courses were given intravenously in a dosage of 10 mg/m² with a median 3 weeks' interval (range 2-11). Mitomycin C was dissolved in 100 ml

SURVIVAL

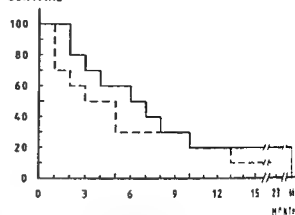


Fig. 2. Survival (in per cent) from time of diagnosis (—) and beginning of mitomycin C treatment (---).

isotonic sodium chloride solution and infused in 30 min together with a fast running infusion of 100 ml isotonic sodium chloride solution. The dose was reduced according to myelosuppression (Table 1).

The response was assessed according to the following criteria. Stationary disease when the measurable tumor decreased or increased with no more than 50 per cent of the initial size for at least one month. Progressive disease when the tumor increased more than 50 per cent of the initial size or a new lesion appeared and treatment failure when patients died after one course of mitomycin C.

Results

Stationary disease was obtained in 4 patients and progressive disease occurred in 4 and treatment

Table 1

Data of the cases before mitomycin C treatment

Case No.	Sex	Age	Performance status	Initial surgery	Stage of primary tumor
1	F	48	II	C+ICR	V
2	F	55	III	E	V
3	F	69	I	C	V
4	F	55	I	C	III
5	F	49	I	C+LNE	III
6	F	65	I	F	V
7	M	64	I	C+CD+GI	V
8	F	77	II	C	V
9	M	59	III	E	V
10	F	78	II	C+CDT	V

C=cholecystectomy, E=explorative laparotomy, ICR=ileocolic resection, LNE=lymph node excision, CDT=choledochotomy.

Table 2

Scheme for dose modification of mitomycin C

Leukocytes ($\times 10^9$ cells/l)	Platelets ($\times 10^9$ /l)	Per cent of planned dose
3-1-3-5	71-100	50
2-1-3-0		25
≤ 1 0	≤ 70	0

failure in 2 patients. Eight patients with gallbladder carcinoma stage V survived for a median 3 months after mitomycin C treatment. 2 with stage III survived 13 and 23+ months respectively (Table 3 Fig. 2).

After the intraarterial infusions one patient had a local hematoma in the groin. 5 patients had a mild leukopenia and one had a longlasting thrombocytopenia. Otherwise no severe side effects of the intraarterial treatment occurred.

After intravenous mitomycin C treatment 4 patients had a mild leukopenia for a short time and 4 patients had thrombocytopenia, extreme and longlasting in one of these. Nausea, diarrhea and extensive alopecia occurred in one patient and severe gastrointestinal bleeding in one. No side effects from other organ systems were noted. No drug related deaths occurred.

Discussion

Mitomycin C has proved to be effective in several cases of gallbladder carcinoma (SHIRAHARA GUERRO et coll. MOERTEL SHIGENAGA). However in the present series no objective remission occurred in 10 cases of advanced gallbladder carcinoma. The result was statistically significantly lower ($p=0.002$ binomial test) than the 47 per cent objective remission rate in a compiled review of cases published by CROOKE & BRADNER (1976). 7 patients with objective remission of 15). The present result did not differ ($p=0.31$ binomial test) from another series of cases with an 11 per cent objective remission rate (3 cases of 27 SHIRAHARA).

In 2 cases myelosuppression was so severe that the mitomycin C treatment was discontinued. This corresponds to previous reports (CROOKE & BRADNER). No drug related deaths occurred. Thromboembolism was not encountered in any case which is in accordance with previous experiences of intraarterial mitomycin C treatment at this hospital (MATSSON et coll. 1977, HELLEKANT & SVANBERG 1978, HELLEKANT et coll. 1978). This may be due to the duration of infusion. In long term intraarterial infusions thromboembolism was frequent (CLOUSE et coll. 1977).

Patients with advanced gallbladder carcinoma have an extremely poor prognosis. Radical surgery does not improve survival compared with cholecys-

Table 3

Doses and results of mitomycin C in gallbladder carcinoma

Case No	Mitomycin C treatment				Overall total dose (mg/m ²)	Response	Survival (in months)	
	Intraarterially		Intravenously				After diagnosis	After mitomycin C
	No of courses	Total dose (mg/m ²)	No of courses	Total dose (mg/m ²)				
1	-	-	1	10	10	TF	7	1
2	-	-	1	10	10	TF	-	1
3	3	78	7	52	80	NC	10	10
4	3	37	5	33	65	NC	35+	73+
5	3	77	15	67	89	NC	66	13
6	3	30	-	-	30	PD	4	3
7	3	17	3	13	30	NC	11	5
8	-	-	6	53	53	PD	8	5
9	2	16	-	-	16	NC	7	1
10	5	41	-	-	41	PD	3	2

NC=stationary disease PD=progressive disease TF=treatment failure

tectomy (DOWDY 1969) Single drug chemotherapy with 5 fluorouracil has proved to be of no use (MOERTEL PERPETUO et coll.) High voltage ir radiation has given alleviation (MOERTEL SMORON 1977 KOPELSON et coll 1977) combination chemotherapy with 5 fluorouracil and nitrosourea or adriamycin has given objective remission (PERPETUO et coll.) in small series of patients

The median survival in the present series was longer than in previous reports. Thus 8 patients with carcinoma stage V survived for a median 5 months after diagnosis. In other series of all stages of gallbladder carcinoma treated with or without chemotherapy median survival was 3 to 5 months (MOERTEL PERPETUO et coll.) Two patients with carcinoma stage III who had a relapse after nonradical surgery survived longer after mitomycin C treatment (13 and 23+ months) than the patients with stage V (median survival 3 months). The prolonged survival may be due to less extensive or less malignant tumor or to a better effect of mitomycin C. No objective remission occurred in the present series but the survival was prolonged compared with previous reports. Therefore further trials with mitomycin C in gallbladder carcinoma are indicated.

SUMMARY

Seven patients with advanced gallbladder carcinoma were given intraarterial infusions of 10 to 15 mg mitomycin C with 10 to 24 days interval. 3 received intravenous infusion (10 mg/m²) with median 3 weeks interval. Stratum disease was obtained in 4 patients, progressive disease in 4 and treatment failures in 2. The median survival after mitomycin C treatment was 4 months (range 1-23+). Irreversible thrombocytopenia occurred in 2 patients and a severe gastrointestinal bleeding episode in one.

REFERENCES

- CLOUSE M F, AHMED R, RYAN R B, OBERHILDER A and McCARTHY J A. Complications of longterm transbiliary hepatic arterial infusion chemotherapy. *Amer J Roentgenol* 129 (1977) 799.
- CRONIN S T and BRADNER W T. Mitomycin C: A review. *Cancer Treat Rev* 3 (1976) 121.
- DOWDY G III JR. The biliary tract. Lea and Febiger Philadelphia 1969.
- GUERRO R C, ABELLO E, CUSTODIO D and SAND E. Chemotherapy of cancer mitomycin C. *J Pharmacol Med* 48 (1972) 559.
- HELLEKANT C and SVANBERG L. Bronchial artery infusion of mitomycin C in advanced bronchogenic carcinoma. *Acta radiol Oncology* 17 (1978) 449.
- BOUSEN E and SVANBERG L. Preoperative intra-arterial mitomycin C in the bronchial artery in squamous carcinoma of the lung. *Acta radiol Diagnosis* 19 (1978) 1045.
- KOPELSON G, HARISIADIS L, TRETTER P and CHIN H. The role of radiation therapy in cancer of the extrahepatic biliary system. An analysis of thirteen patients and a review of the literature of the effectiveness of surgery, chemotherapy and radiotherapy. *Int Radiat Oncol Biol Phys* 2 (1977) 883.
- MATTSSON W, HELLEKANT C and ANDREASSON I. Combination chemotherapy of advanced squamous carcinoma of the head and neck. *Acta radiol Ther Phys Biol* 16 (1977) 385.
- MOERTEL C G. The gallbladder. In: *Cancer medicine* 1547. Edited by J F Holland and E Frei III. Lea Febiger Philadelphia 1973.
- NEVIN J E, MORAN T J, KAY S and KING R C. Carcinoma of the gallbladder: Staging, treatment and prognosis. *Cancer* 37 (1976) 141.
- PERPETUO M D, C M O, VALDIVIESO M, HENRIKSEN K, NELSON R E, CONNOR T and BODEY G. Natural history of gallbladder cancer: A review of 10 years experience at M D Anderson Hospital. *Tumor Institute Cancer* 42 (1978) 330.
- SHICENAGA K. Effect of mitomycin C on gallbladder cancer, particularly from a histopathological point of view. *J Kumamoto Med Soc* 40 (1976) 97.
- SHIRAHATA Y. Methods of drug therapy in cancer. *J Cancer Clin* 14 (1968) 191.
- SMORON G L. Radiation therapy of carcinoma of the gallbladder and biliary tract. *Cancer* 40 (1977) 144.
- WARREN K W, HARDY K J and O'ROURKE M G. Primary neoplasia of the gallbladder. *Surg Gynecol Obstet* 126 (1968) 1036.
- ZUBROD C G, SCHINDLERMAN M, FREI E I, BRINDLEY C, GOLD L, SHINDLER D, O'NEILL GORMAN J, JONES R JR, JONSSON U, CHAMBERS T, CHALMERS T, FERGUSON B, DEDERICK A H, LIND J, SHAWRY O, RICHESON W, LASKIN and OWENS A H JR. Appraisal of method and study of chemotherapy of cancer in man. Comparative therapeutic trial of nitrosourea mustard and thiophosphoramide. *J Chron Dis* 13 (1960).

FROM THE DEPARTMENTS OF NUCLEAR MEDICINE AND OF ONCOLOGY AND RADIATION THERAPY THE RADIUM CENTRE AARHUS KOMMUNEHOSPITAL AND UNIVERSITY OF AARHUS DK 8000 AARHUS DENMARK

PENTAGASTRIN, CALCIUM AND WHISKY STIMULATED SERUM CALCITONIN IN MEDULLARY CARCINOMA OF THE THYROID

K. EMMERTSEN, H. B. NIELSEN, L. MOSEKILDE and H. HVID HANSEN

Medullary thyroid carcinoma (MCT) is a tumour of the calcitonin secreting parafollicular C cells of the thyroid gland. The disease may be familial with an autosomal dominant mode of inheritance and often associated with other endocrine neoplasms.

Determination of serum immunoreactive calcitonin (SiCT) concentrations is the most sensitive method for the diagnosis (TELENIUS BERG *et coll* 1975) but basal levels may be within normal range in cases of C cell hyperplasia and small carcinomas. However, calcitonin secretory capacity is abnormally high in these patients. Determination of SiCT concentrations after stimulative procedures can therefore disclose the C cell neoplasm in an early stage (SIZEMORE & GO 1975, MILHAUD *et coll* 1975, TELENIUS BERG *et coll* 1977, HILLYARD *et coll* 1978, GRAZE *et coll* 1978).

Intravenous injection of pentagastrin is now the most commonly used stimulative procedure (TELENIUS BERG *et coll* 1977, GRAZE *et coll*) but superiority has been claimed for the use of short term calcium infusion (RUDE & SINGER 1977) or oral whisky (DYMLING *et coll* 1976, HILLYARD *et coll*). Therefore, these three stimulative procedures for calcitonin secretion were compared in patients with proven MCT and in healthy controls.

Material and Methods

The material comprised 6 patients (3 males and 3 females, aged 30–65 years) all previously operated

upon for MCT, microscopically proven and with persistently elevated basal SiCT concentration and 8 healthy volunteers (4 males and 4 females, aged 24–40 years). MCT was familial in one (Fig. 3 E, J) and sporadic in the other 5 patients.

Serum concentrations of calcium, phosphorus, alkaline phosphatase and creatinine were within normal limits in all subjects. Informed consent was obtained from all individuals.

SiCT was measured in all subjects before and 2, 5, 15 and 30 min after stimulation with pentagastrin (Peptavlon 0.5 µg per kg body weight diluted in 1 to 2 ml isotonic sodium chloride given i.v. within 5 s), calcium (2 mg per kg body weight as calcium laevulatis 10% given intravenously during 1 min) and whisky (50 ml Johnny Walker Black Label by mouth). The order of the tests differed between the subjects. During calcium infusion SiCT and serum calcium were measured simultaneously. In each individual the calcitonin stimulation tests were initiated at 11.00 a.m. after fasting overnight. The injections were given and the blood samples drawn through an intravenous catheter.

SiCT was measured by radioimmunoassay as described previously (NIELSEN *et coll* 1979, NIELSEN & OLSEN 1979) using a commercial antibody to synthetic human calcitonin (Calbiochem, U.S.A.) and synthetic human monomer calcitonin (Ciba, Switzerland) for standards and iodination. The de-

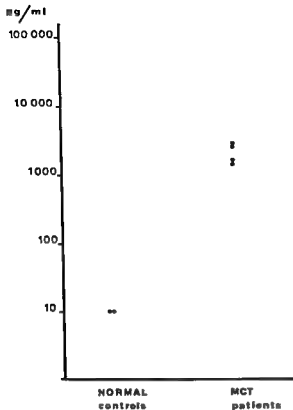


Fig 1

tection limit was 20 pg/ml. Normal range was 0 to 120 pg/ml and detectable serum values could be found in 65 per cent of normal subjects. The intraassay coefficient of variation was below 3 per cent at a serum concentration of 350 pg/ml and 14 per cent at a serum concentration of 30 pg/ml. The interassay variation coefficient was 14 per cent at a S-CT concentration of 140 pg/ml.

Statistical evaluation: A significant increase in S-CT was defined as an increase exceeding twice the analytical error. The relative increases in S-CT were compared using Student's *t* test for paired comparisons.

Results

The basal S-CT values in MCT patients and normal controls are given in Fig 1. S-CT was increased in all patients with MCT, ranging from 380 to 120,000 pg/ml. S-CT was undetectable in 5 of the 8 volunteers and within normal limits (less than 120 pg/ml) in the rest.

The maximum increases in S-CT during stimulation with pentagastrin, calcium and whisky in the normal controls appear in Fig 2. A significant increase in S-CT ($p < 0.05$) was found in 2 controls following pentagastrin, in 3 following calcium and in

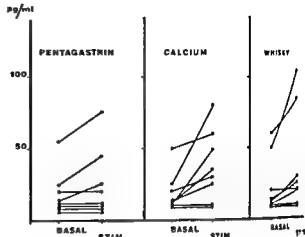


Fig 2

Fig 1 S-CT (pg/ml) in 8 patients with medullary carcinoma of the thyroid (MCT) and in normal controls.

Fig 2 S-CT (pg/ml) in 8 healthy individuals before and after (maximum value) stimulation with pentagastrin 0.4 µg/kg body weight, calcium 2 mg/kg body weight and whisky 40 ml.

3 following whisky. The maximum increase in S-CT was found 2 to 15 min following stimulation with whisky. There were no significant differences between the stimulation procedures. None of the stimulated values exceeded the upper normal basal range of 120 pg/ml. The mean rise in serum calcium during calcium infusion was 1.4 mmol/100 ml (range 1.0–1.7).

The effects of stimulation with pentagastrin, calcium and whisky on S-CT in the 6 patients with MCT appear in Fig 3. A significant increase in S-CT ($p < 0.05$) was found in all 6 patients following pentagastrin, in 5 patients following calcium and in 4 patients following whisky. The maximum increases in S-CT were found 2 to 5 min following stimulation without differences between the stimulation procedures.

The average absolute increase in S-CT was 8150 pg/ml (range 2150–100,000) following pentagastrin, 23,400 pg/ml (range 0–115,000) following calcium and 13,800 pg/ml (range 0–80,000) following whisky. The relative mean increase in S-CT was 8 per cent (range 1.30–1.475) after pentagastrin, 44 per cent (range 0–686) after calcium and 18 per cent (range 0–89) after whisky. The mean rise in serum calcium during calcium infusion was 1.3 mmol/100 ml (range 0.4–1.9).

The greatest increase in S-CT occurred in 4

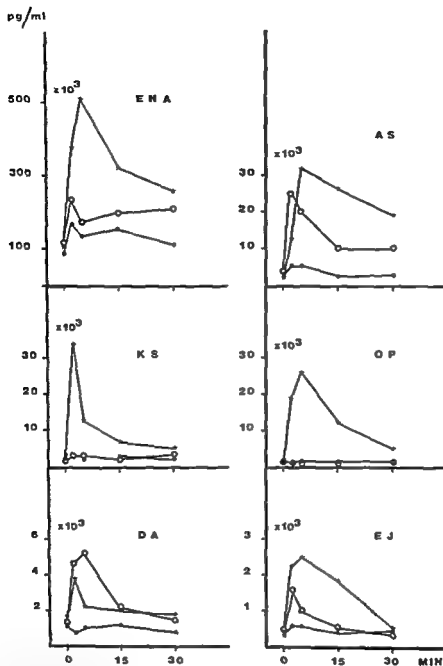


Fig. 3 S iCT (pg/ml) in 6 patients with medullary carcinoma of the thyroid before and after stimulation with pentagastrin (\circ) 0.5

μ g/kg body weight calcium (\circ) 2 mg/kg body weight and whisky (\bullet) 50 ml

tients after stimulation with pentagastrin and in one patient after stimulation with calcium. No significant difference was found between the relative increase in S iCT following stimulation with pentagastrin or calcium. However, the relative increase in S iCT following whisky was significantly ($p = 0.02$) lower than following pentagastrin, where is no significant difference was found between the increases in S iCT following whisky and calcium.

Discussion

The results showed that all 6 patients with MCT responded to pentagastrin with a marked increase in S iCT, while 5 patients responded to short term calcium infusion and 3 to oral whisky. The maximum stimulated S iCT values were found after pentagastrin in 5 patients and after calcium in one patient. Among the 11 normal controls only a modest increase in S iCT after pentagastrin, calcium and whisky was

found with no difference between the three procedures.

In several reports on screening for familial MCT (SIZEMORE & GO TELENUS BERG et coll 1977, GRAZE et coll.) it was stated that pentagastrin 0.5 µg/kg body weight given intravenously as a bolus is a potent stimulator for iCT secretion in patients with C cell neoplasms, an excessive response being the most diagnostic. On the other hand RUDE & SINGER showed that short term infusion of calcium 2 mg/kg body weight during one minute was a more potent stimulator for iCT secretion than pentagastrin in 4 patients with MCT. In this series one patient did not respond at all to pentagastrin.

A 4 hour infusion of calcium 15 mg/kg body weight was previously the standard stimulative procedure for disclosing the abnormally high S iCT response in patients with C cell neoplasms. However this method is time consuming, frequently causes nausea and vomiting and gives no advantage compared with pentagastrin (TELENUS BERG et coll 1975, SIZEMORE & GO, GRAZE et coll.).

Injection of pentagastrin is followed by a sudden onset of generalized unpleasantness with oppression and abdominal discomfort lasting for about 2 min. Short term calcium infusion produces less severe side effects, but may give nausea and a general feeling of warmth.

As a convenient and well tolerated stimulative procedure oral whisky has been introduced. Ingestion of 50 ml whisky increased S iCT by a mean of 185 per cent in 14 patients with MCT (DYMILING et coll.). This method was subsequently used for detection of familial MCT (DYMILING et coll., HILL, YARD et coll.). In these series patients with basal S iCT concentrations within normal range and abnormally high S iCT responses to whisky did not undergo thyroidectomy with microscopic examination for C cell neoplasms. Thus the frequency of true and false positive reactions could not be determined. Likewise when evaluating stimulative procedures in family members at risk with borderline elevation of S iCT, the frequency of false negative reactions cannot be evaluated unless all family members undergo thyroidectomy. Therefore the three stimulative procedures were tested in patients with known C cell neoplasms, assuming that results obtained in these patients are valid also in patients with small C cell neoplasms and borderline elevated S iCT concentration. Using the iCT assay whisky was a weak and unreliable stimulator for S iCT

compared with pentagastrin in 6 patients preoperated upon for MCT and with elevated S iCT concentrations, indicating persistent C cell neoplasia. Similar results have been obtained by TELENUS BERG et coll. (1977).

In screening for familial MCT intravenous pentagastrin is recommended as the stimulative agent with measurement of S iCT concentrations before and 2 and 5 min after injection. Considered the single patient with MCT of RUDE & SINGER who did not respond to pentagastrin, short term calcium infusion should be carried out if results obtained after pentagastrin are equivocal or if clinical suspicion persists despite a negative S iCT response to pentagastrin.

SUMMARY

The efficiency of pentagastrin, calcium and whisky in raising serum immunoreactive calcitonin (S iCT) concentrations was analysed in 6 patients with medullary carcinoma of the thyroid and in 8 healthy controls. All patients responded to pentagastrin with a significant increase in S iCT. 5 responded to calcium and only 1 to whisky. In the 8 controls no or only a modest increase in S iCT occurred following pentagastrin, calcium and whisky with no difference between the three. It is concluded that pentagastrin is the most useful stimulative agent for iCT secretion in patients with C cell neoplasms. In selected cases the additional use of calcium could be advantageous.

ACKNOWLEDGEMENTS

Synthetic human monomer calcitonin was kindly donated by Ciba, Switzerland. The investigation was supported by grants from the Danish Medical Research Council (Nos. 512 10195 and 512 15513).

REFERENCES

- DYMILING J F, LUNDBERG O, HILLIARD C J, GALT BRIC P B, EVANSI M A and MACINTYRE I W. Whisky: a new provocative test for calcitonin secretion. *Acta endocrin* (Kbh) 82 (1976) 500.
- GRAZE K, SHILLER J J, TASHIRO A H, ABRAHAM E W, CIRVSKINER S, GARDNER J, MILLER R P, WOLFE H J, DILLON R A, TAYLOR H, HILLIARD C J and RICHES S. Natural history of familial medullary thyroid carcinoma: Effect of a preoperative diagnosis. *New Engl J Med* 199 (1976) 9.
- HILLIARD C J, EVANSI M A and HILL P A. Familial medullary thyroid carcinoma. *Lancet* 119 (1976) 119.
- MILHAUD G, RICHES S, CALMEIN C, TOU J, COLETTIS G et MOUKHTAR M S. Effect of stimulation of the secretion of calcitonin in certain cancers medullaires de la thyroïde. *Soc méd* 4 (1975) 1793.

- ELSEN H E and OLSEN K J Serum calcitonin after renal transplantation Acta med scand 205 (1979) 619
- CHRISTENSEN C K and OLSEN K J Serum calcitonin in patients with chronic renal disease Acta med scand 205 (1979) 615
- DE K K and SINGER F R Comparison of serum calcitonin levels after a 1 minute calcium injection and after pentagistatin injection in the drignosis of medullary thyroid carcinoma J Clin Endocr Metab 44 (1977) 980
- SIZEMORF G W and GO V L W Stimulation tests for diagnosis of medullary thyroid carcinoma Mayo Clin Proc 50 (1975) 53
- TELENUS BERG M ALMQUIST S and WASTHED B Serum calcitonin response to induced hypercalcemia Acta med scand 197 (1975) 367
- — BERG B HEDNER P INGEMANSEN S TIBBLIN S and WASTHED B Screening for medullary carcinoma of the thyroid in families with Sipple's syndrome Evaluation of new stimulation tests Europ J Clin Invest 7 (1977) 7

NEUTRON RADIATION THERAPY OF PAROTID GLAND TUMORS

J P GERACI

The first report on neutron treatment of tumors arising from the parotid gland appeared in 1942 (STONE & LARKIN). By 1948, 3 of 9 patients treated with neutrons were still alive but all had distressing late effects (STONE 1948). All 3 survivors had trouble with jaw movement, skin atrophy, absence of subcutaneous fat and subcutaneous induration. One had an ulcer during the third year after treatment which healed. One developed radiation osteitis and draining of the sinus during the sixth year after treatment. Since STONE recognized that the quality of survival is as important as the length of survival, he discouraged further use of neutrons for treating parotid tumors as well as other malignant tumors.

Following STONE's clinical trial, FOWLER & FORGAN (1963) showed that the relative biologic effectiveness (RBE) of neutrons relative to photons increases with decreasing neutron dose per fraction. This led to the realization that STONE may have used excessively high doses by relying on RBE measured in experimental animals at large neutron doses to determine the dose equivalency of neutrons and photons at lower clinical neutron doses per fraction. This resulted in renewed interest in neutron therapy and preliminary clinical investigations were undertaken in England in the late sixties and later on in the United States.

HENRY et coll (1978) and CATTERALL (1980) recently have published their results for neutron therapy of salivary gland tumors. CATTERALL found that 30 of 34 patients had complete tumor regression. In contrast to the results of STONE, CATTERALL reported only 7 complications, 4 of which

completely recovered. HENRY et coll reported that neutron therapy resulted in 100 per cent local control rate for tumors less than 3 cm, 75 per cent for those between 3 and 6 cm, and no local control rate for tumors larger than 6 cm in diameter. Similar to the results of CATTERALL, HENRY et coll reported no long term complications at the time their results were presented.

Unfortunately, in none of these reports sufficient information is given with respect to individual treatment protocol, tumor site (i.e. parotid, submaxillary, etc.), tumor dose, tumor size, regional node involvement, follow up time, or survival time to make a meaningful comparison with the neutron treated patients of STONE or with patients treated with conventional photons or surgery. The late effects noted by STONE were based on patients with parotid tumors who had been followed up for 60, 75 and 92 months. Therefore, a critical question is how many patients in the current neutron trials have had comparable follow up times to develop similar late effects. In this regard, a special opportunity exists at the University of Washington where 9 patients with parotid gland tumors have been treated with neutrons. It has been possible to compare these neutron results with those of STONE, as well as with published results involving photon irradiation and surgical treatment of parotid tumors. Furthermore, in the Seattle area, a central tumor registry exists, referred to as the Cancer Surveillance System at the Fred Hutchinson Cancer Research Center. A particularly

Submitted for publication 15 October 1979

Table I
Extent of disease codes for the parotid gland

	Local vessel invasion	Regional lymph nodes	Size of primary tumor			
			2 cm or less	2.1 to 3.9	4 cm or more	Subtotal
Primary tumor: no direct extension, no distant involvement						
Single focus and entirely within benign tumor capsule	No	No	10	15	20	25
Substance of parotid gland invaded	No	No	11	16	1	28
Confined to substance of parotid gland but multicentric foci	No	No	12	17	2	31
Localized: no detailed information	No	No	14	19	24	57
Single focus and entirely within benign tumor capsule	Yes	No	30	35	40	105
Substance of parotid gland invaded	Yes	No	31	35	41	107
Confined to substance of parotid gland but multicentric foci	Yes	No	32	37	4	73
Localized: no detailed information	Yes	No	34	39	44	117
Single focus and entirely within benign tumor capsule		Yes	40	55	60	155
Substance of parotid gland invaded		Yes	51	56	61	168
Confined to substance of parotid gland but multicentric foci		Yes	52	57	62	171
No detailed information of above		Yes	54	59	64	177

Regional lymph nodes for this site (first chain of drainage): parotid gland group

useful feature of this tumor registry is that for most patients with malignant tumors the extent of the disease is expressed in a two-digit code (Tables 1-2). Since the coding is done by experienced tumor registrars at each participating hospital, a third-party evaluation of the extent of disease for each patient is available. This information also allows selection of control patients with the same severity of the disease as the neutron-treated patients but who were treated with photons. As a result it has been possible to make a preliminary evaluation of the efficacy of neutrons in treating parotid gland tumors based on a retrospective analysis of the clinical data for photon and neutron-treated patients.

Method

The 9 patients with parotid gland tumors were treated with neutrons between May 1974 and January 1977. The patient charts were reviewed to obtain the treatment protocol (radiation alone or

surgery plus radiation), type of irradiation (neutron or combined neutron/photon), tumor size and size tumor type, nature of disease (primary or recurrent), follow-up time, survival time and complications. The extent of disease code was available for 6 cases from the Cancer Surveillance System Registry. The other 3 cases were retrospectively based on the information in the patients' records. Photon-treated patients in the Cancer Surveillance System who had the same type of tumor and extent of disease were selected as controls. Data for neutron-treated patients were also retrospectively staged using the staging system described by et coll. (1977).

Patients were treated with neutrons produced by bombardment of the beryllium target with 2.5 MeV deuterons at the University of Washington neutron beam. Depth dose and skin sparing properties of the neutron beam are similar to those for ^{60}Co gamma rays. Physical doses were measured in equivalent ionization chambers. Patients re-

Table 2

Extent of disease codes for the parotid gland

	Involvement of regional lymph node	
	No	Yes
Limited direct extension		
Penetration of capsule of gland into connective tissue	70	80
Involvement of		
neural sheath (facial nerve)	71	81
muscle	72	82
skin	73	83
periosteum of mandible	75	85
pharyngeal mucosa	76	86
more than one (70-76) or (80-86)	79	89
Further direct extension		
Ulceration of skin	90	-0
Mandible	91	-1
Skull	92	-2
External auditory meatus	93	-3
Major blood vessels	95	-5
More than one (90-95) or (-0 through -5)	99	-9
Distant involvement		
Distant site	Δ1	Δ6
Distant lymph node	Δ2	Δ7
Distant site and distant lymph node	Δ3	Δ8
Regional lymph nodes for this site (first chain of drainage) parotid gland group		

only neutron treatment were treated twice (Monday and Friday) or 4 times a week (Monday, Tuesday, Thursday and Friday). Patients receiving combined neutron/photon irradiation were treated twice a week with neutrons (Monday and Friday) and 3 times a week with photons (Tuesday, Wednesday and Thursday). The total irradiation times were between 6 and 8 weeks. The total dose was calculated by multiplying the neutron dose by the RBE of 3.0 which was used clinically (GRIFFIN et al 1980) and adding that figure to the dose delivered by photons.

Survival rates were determined by the life table method of MACDONALD (1962).

Results

The profile of the 9 neutron treated patients with respect to the severity of disease and treatment characteristics is presented in Table 3. Seven of the patients had primary tumors and 6 of these had

either most or all of the parotid tumor resected before the neutron irradiation. The 3 patients receiving neutron therapy alone had persistent or recurring tumors following treatment. These 3 patients also had the largest tumors in the series and 2 had regional node involvement. The tumor type in the majority of the patients was either adenoid cystic carcinoma or mucoepidermoid carcinoma. Seven of the patients are now deceased but one who had the least severe disease has a survival time of 5 years.

The profile of 6 photon treated patients obtained from the Cancer Surveillance System registry who had equal or greater severity of disease in comparison to the neutron treated patients as indicated by the extent of disease code is presented in Table 4. Of these 6 patients 3 had regional node involvement and 5 had adenocarcinoma. Five patients had been operated upon before irradiation. Three of these patients are deceased.

The normal tissue complications noted in the patients' records for neutron treated patients surviving

Table 3
Neutron treated parotid gland tumors

Case No	Disease	Tumor size (cm) ^a	Node size (cm) ^a	Tumor type	EOD ^b Stage ^c	Treatment ^d	Dose ^e	Tumor status ^f	Vital status	Follow up time (mo) ^g
1	Primary	7	0	Adenoid cystic carcinoma	31 I	Surgery + mxRT	67.0	NED	Alive	60
2	Primary	4	0	Mucoepidermoid carcinoma	99 III	Surgery + mxRT	58.5	M	Dead	10
3	Recurrent	15	0	Mucoepidermoid carcinoma	&1 IV	nRT	55.5	P+M	Dead	9
4	Primary	4	0	Adenocarcinoma	91 III	Surgery + mxRT	63.7	M	Dead	74
5	Recurrent	7	3	Acinic cell carcinoma	-9 IV	Surgery + nRT	60.0	R	Dead	40
6	Primary	9.5	0	Adenoid cystic carcinoma	71 III	nRT	60.0	R	Dead	13
7	Primary	4	2	Squamous cell carcinoma	&3 IV	Surgery + nRT	56.0	M	Dead	17
8	Primary	8	0	Undifferentiated carcinoma	99 III	nRT	64.5	P	Dead	13
9	Primary	4	0	Adenoid cystic carcinoma	71 III	Surgery + mxRT	64.8	NED	Alive	8

Tumor size measured at the largest dimension

Extent of disease (EOD) codes were obtained from the Cancer Surveillance System survey and are defined in Tables 1 and 2

6 and 8 were uncoded and therefore were retrospectively coded based on the information in the patient records

Diseases are retrospectively staged based on the staging system used by Fu et al

^d mxRT indicates mixed neutron and photon irradiation nRT indicates neutron treatment

^e Doses reported are in Gy and were calculated by multiplying the neutron dose by the RBE of 3 which was used clinically (Griffith et al) and adding the dose delivered by photons

^f Tumor status at last follow up NED — No evidence of disease R — Recurrent local disease I — Persistent local disease M — Metastatic diseases

^g Follow up times are measured from the date of diagnosis The follow up times of deceased patients are the actual survival times

Table 4
Fluorine treated parotid gland tumors

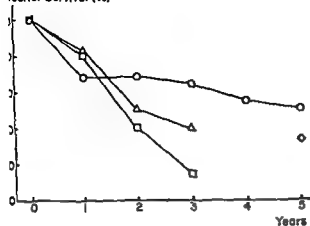
Case No	Tumor type	EOD ^a	Treatment	Vital status	Follow up time (mo) ^b
10	Adenocarcinoma	&1	RT	Dead	18
11	Mucoepidermoid carcinoma	81	Surgery + RT	Alive	58
12	Adenocarcinoma	&1	Surgery + RT	Dead	23
13	Adenocarcinoma	81	Surgery + RT	Alive	76
14	Adenocarcinoma	89	Surgery + RT	Dead	8
15	Adenocarcinoma	73	Surgery + RT	Alive	25

All cases were obtained from the Cancer Surveillance System

Extent of disease (EOD) codes were obtained from the Cancer Surveillance System survey and are defined in Tables 1 and 2

Follow up times are measured from date of diagnosis The follow up times of deceased patients are the actual survival times

Survival (%)



actuarial survival of neutron and conventionally treated patients with advanced carcinoma of the parotid gland. Error bars are omitted for clarity. O Conventional therapy stage III (FU et al 17 patients); Δ Conventional therapy all cases from Cancer Surveillance System (present report 6 patients Table 4); Stone (9 patients); □ Neutron therapy primary tumors stage III (present report 5 patients Table 3). The 3 year survival \pm SD are 61.3 ± 10.2 , 40.0 ± 11.9 , 33.3 ± 11.1 and 11.1 ± 8 respectively.

more than 2 years are presented in Table 5. In 3 of 4 cases evidence of significant alterations in normal tissues (primarily the skin) within the treatment volume was found. Of particular interest is Case No. 1 who had a parotid tumor on the left side and has the longest survival time to date. Significant fibrosis, telangiectasia and a 5 mm ulceration developed in the treated area 58 months after treatment. At 60 months after the irradiation this patient developed headache, intermittent right body seizures with aphasia and left eye pain.

The actuarial survival curve for the 5 neutron treated primary tumors stage III (Cases 2, 4, 6, 8, 9 Table 2) appears in the Figure. For comparison the survival curve for 22 patients stage III treated with conventional therapy (surgery, photons, or a combination thereof) reported by Fu et al, as well as a curve for all photon treated patients obtained from the Cancer Surveillance System (Table 3) are also plotted. The results show that the survival rate one year after treatment for the 5 neutron treated patients is similar to those who received conventional treatment. However, at longer times after treatment conventionally treated patients had a better survival rate than the neutron treated patients. In addition, the long term survival of the neutron treated patients is not better than for the incurable patients treated by Stone in the 1940's.

Table 5

Neuronal tissue complications for neutron treated patients with a follow-up time of at least 2 years

Case No.	Complications	Approximate time of occurrence (mo.)
1	Significant fibrosis, telangiectasia and 5 mm ulceration in the treated area	58
	Headaches, intermittent right body seizures with aphasia and left eye pain	60
4	Bone	
5	Neck with very marked areas of necrosis	30
	Several small ulcerations in treated area	33
9	Local pain, dryness of mouth and tissue thickening in treated area	25

Case number refers to cases in Table 3.

Complications are those expressed in the patients' records. Times measured are from date of diagnosis.

Discussion

HENRY et al¹ and CATTERALL² used local control as a basis for conclusion of results. In the present series this basis is patient survival. STONE & LARKIN³ in their initial clinical neutron trials recognized the importance of survival as a basis for conclusion when they stated: "the ultimate aim of all treatment is the survival of the patient and the result of (neutron author's insertion) treatment must be judged by this criterion." However, in considering the results of the present series in terms of survival it is important to recognize that this is a preliminary evaluation of the efficacy of neutron therapy of parotid gland tumors since it is based on a small number of patients in a non-randomized trial to conclusively prove or disprove the superiority of neutrons. Randomized clinical trials involving more patients are needed. Nevertheless, it is of value to estimate the trend expected in this renewed treatment as soon as possible and in particular to compare the early neutron results of STONE with these more recent efforts.

Unfortunately, the clinical results in the present series indicate no improvement in survival rate for neutron treated primary parotid gland tumors stage III as compared with conventional treatment (Figure). This is in spite of the fact that none of the 5 neutron treated patients had regional node involvement whereas one half of the patients in the Cancer

Surveillance System (Table 4) and one third in the series of FU et coll. did have regional node involvement. Furthermore, a possible favorable bias towards neutrons in this comparison is indicated in that the prognosis with regard to survival for the tumor types (i.e. adenoid cystic and mucoepidermoid carcinoma) in most of the neutron treated patients on the whole is better than that for adenocarcinoma (FU et coll.). The tumor type in 5 of 6 photon treated patients (Table 4). It is also of interest that with a maximum follow up time of less than 5 years only 2 of the present 9 patients treated with neutrons are presently alive (Table 3). This result is not better than that of STONE who reported 3 of 9 patients surviving longer than 5 years.

An argument frequently expressed for the poor survival results obtained is that the disease has been so extensive in the neutron treated patients that it is unlikely that any therapeutic method could control the tumors. Therefore, the biologic advantages of fast neutrons will only be evident by treating less extensive malignant tumors (PARKER et coll. 1977; CATTARALI et coll. 1977). Presumably this would mean carcinoma stage II of the parotid gland. First of all, it is noteworthy that in 1942, when only the early effects were evident, STONE & LARKIN expressed a similar sentiment when they stated: therefore, it should now be permissible to treat small cancer lesions that are more likely to be cured than the extensive ones. However, more important, patients with malignant tumors of the parotid gland stage II have a 5 year survival rate of approximately 75 per cent with conventional therapeutic methods (FU et coll.). Therefore, to detect a 15 per cent increase in survival after neutron treatment (i.e. from 75 to 90%) at a significant level of 5 per cent with a probability of detection of 80 per cent would require 76 patients being treated with conventional methods and the same number of patients treated with neutrons (COCHRAN & COX 1957). The scarcity of parotid gland tumors stage II would seem to preclude such a trial.

Of particular importance in the present series is the observation that for 3 of the 4 patients surviving longer than 2 years considerable tissue damage occurred in the treatment volume (Table 5). This is to be expected since WITHERS et coll. (1978) have shown that the neutron RBE for late skin damage in pigs (considered a good animal model for human skin) is 3.1 to 3.4 for a 50 MeV deuteron produced neutron beam. Correcting this RBE for the 20 per

cent greater biologic effect of the University of Washington 22 MeV deuteron produced neutron beam (HALL 1977) results in a RBE for late skin damage of 3.7 to 4.1. Since the RBE of 3.0 was clinically for calculating the equivalence of neutron and photon doses, this would mean that the patients received approximately a 10 to 36 per cent overexposure to skin. Since the dose response curve for late complications in the head and neck region in humans is steep (SHUKOVSKY 1970; HERRING 1976), such an increase in radiation dose should substantially increase the late normal tissue complication rate. Therefore, lower tumor doses may be necessary in future neutron clinical trials to avoid late complications. However, it should be recognized that in reducing the doses the probability of cure with neutrons is also reduced.

Finally, the higher local control rates reported by CATTARALI for salivary gland tumors treated with neutrons have resulted in these tumors being set out as being encouraging for neutron therapy (FIELD 1976; FOWLER 1976; DUNCAN 1978). Although only the early effects were evident, STONE & LARKIN expressed a similar feeling when they stated: the effects of neutrons on tumors have been such as to encourage further study in selective cases. Surely the parotid gland tumor was one of the cases referred to by STONE & LARKIN since at that time the 3 parotid gland tumors treated were observed to respond unusually well to neutron therapy. In all 3 cases, the tumors disappeared completely after treatment (STONE & LARKIN). Therefore, it remains to be seen if the high local control rate reported for neutron treatment of salivary gland tumors in current clinical trials (HERRING et coll. 1978; CATTARALI) is translatable to higher survival rates with no concomitant increase in late normal tissue complications.

SUMMARY

A retrospective analysis of 9 patients treated with fast neutrons for tumors arising from the parotid gland is presented. In 3 of 4 patients who survived longer than 2 years, significant normal tissue damage occurred within the treatment volume. Five patients treated with neutrons or surgery plus neutrons for primary parotid stage III did not have a better prognosis with respect to survival than a comparable group of patients treated with conventional methods (i.e. surgery plus radiation therapy). These results appear to be in contrast to those obtained for parotid tumors treated with conventional methods in the early 1940's by STONE.

ACKNOWLEDGEMENT

This investigation was supported by Grant No. CA 31 awarded by the National Cancer Institute. DHEW author is grateful to Dr K. L. Jackson for his helpful suggestions and to P. H. Thrower, M. Marino and M. G. Ga for their technical assistance. The assistance of the staffs of the Medical Records Department of the University Hospital and the Cancer Surveillance System of Fred Hutchinson Cancer Research Center is also acknowledged.

REFERENCES

- ATTERALL M. Observations on the reactions of normal and malignant tissues to a standard dose of neutrons. *Europ J Cancer* (1980) In Press.
- BEWLEY D. K. and SUTHERLAND I. Second report on results of a randomized clinical trial of fast neutrons compared with X or gamma rays in treatment of advanced tumors of head and neck. *Brit med J* 1 (1977) 1647.
- CHRISTIAN W. G. and COX G. M. Experimental designs. Second edition. p. 24. John Wiley & Sons, New York, 1957.
- LUCAN W. Current thoughts on fast neutron therapy. *Brit J Radiol* 51 (1978) 943.
- ELD S. B. An historical survey of radiobiology and radiotherapy with fast neutrons. *Curr Topics in Radiat Res Quarterly* 11 (1976) 1.
- WALLER J. F. A review of present needs and future directions. In: Particle radiation therapy, p. 563. Proceedings of an International Workshop, Key Biscayne, Florida, 1975. American College of Radiology, Philadelphia, 1976.
- and MORGAN R. L. Pre therapeutic experiments with the fast neutron beam from the Medical Research Council Cyclotron VIII. General Review. *Brit J Radiol* 36 (1963) 115.
- FLICK K. K., LEIBEL S. A., LEVINE M. L., FRIEDLANDER L. M., BOLES R. and PHILLIPS T. L. Carcinoma of the major and minor salivary glands. Analysis of treatment results and sites and causes of failures. *Cancer* 40 (1977) 2992.
- GRIFFIN T. W., BLASKO J. C. and LARAMORE G. E. Results of fast neutron beam radiotherapy pilot studies at the University of Washington. *Europ J Cancer* (1980) In Press.
- HALL E. J. Radiobiological intercomparisons in vivo and in vitro. *Int J Radiat Oncol Biol Phys* 3 (1977) 195.
- HENRY L. W., BLASKO J. C. and GRIFFIN T. W. Evaluation of fast neutron teletherapy for advanced carcinomas of the major salivary glands. *Int J Radiat Oncol Biol Phys* 4 (1978) Suppl. No. 2, p. 9.
- HERRING D. F. The consequences of dose response curves for tumor control and normal tissue injury on the precision necessary in patient management. *Laryngoscope* 85 (1975) 1112.
- MACDONALD E. J. Method of analysis for evaluation of treatment in cancer of the oropharynx. *Radiology* 79 (1962) 783.
- PARKER R. G., BERRY H. C., CADERAO J. B., GERDES A. J., HUSSEY D. H., ORNITZ R. and ROGERS C. C. Preliminary clinical results from U.S. fast neutron teletherapy studies. *Cancer* 40 (1977) 1434.
- SHILAOVSKY L. J. Dose-time-volume relationships in squamous cell carcinoma of the supraglottic larynx. *Amer J Roentgenol* 108 (1970) 27.
- STONE R. S. Neutron therapy and specific ionization. Janeway Memorial Lecture. *Amer J Roentgenol* 59 (1948) 771.
- and LARKIN J. C. The treatment of cancer with fast neutrons. *Radiology* 39 (1942) 609.
- WITHERS H. R., THAMES H. D., HUSSEY D. H., FLOW B. L. and MASON K. A. Relative biological effectiveness (RBE) of 50 MV (Be) neutrons for acute and late skin injury. *Int J Radiat Oncol Biol Phys* 4 (1978) 601.

FROM THE DEPARTMENTS OF ONCOLOGY (DIRECTOR PROF H KILLANDER) RADIATION PHYSICS (DIRECTOR, PROF K LIDÉN) AND PATHOLOGY (DIRECTOR PROF U STENRAM) THE UNIVERSITY HOSPITAL S-22185 LUND SWEDEN

EFFECT OF DIFFERENT RADIATION FRACTIONATION SCHEDULES ON METASTASES FROM AN OESOPHAGEAL CARCINOMA

C MERCKE I L LAMM P NILSSON T LANDBERG
C H HÅKANSSON and E HAMMAR

The results of radiation therapy of a malignant tumour depend on several factors the most important in clinical routine being the type and size of the tumour the proper delineation of the target volume the size of the total target absorbed dose and its fractionation and the application of chemical or physical agents that may modify the radiation response

Most biologic information is derived from *in vitro* cell systems and experimental animal tumours. These systems cannot be said to reflect the complex *in vivo* conditions of spontaneously appearing tumours in man. The present investigation is an attempt to characterize some inherent biologic properties of a human tumour *in vivo* from the results of irradiation of metastases from an oesophageal carcinoma using different fractionation schedules

Material and Methods

A 70-year old woman with a previous history only of dysphagia developed swallowing disturbances. At radiography a bulging tumour was demonstrated in the oesophagus measuring 8 cm × 4 cm × 4 cm on the films. Biopsy (Fig 1 a b) showed an anaplastic probably epithelial malignant tumour without definite glandular or squamous differentiation. No features of malignant lymphoma or melanoma were found. Assuming the tumour to have the form of a cigar shaped rotation ellipsoid its volume was calculated to be 65 cm³. The patient was considered

inoperable and external irradiation was given using a 6 MV linear accelerator. One anterior and one posterior field (16 cm × 8 cm) were irradiated with beam directions 0° and 180° respectively. One fraction was administered daily for 5 days a week and a total absorbed dose of 40 Gy was given in 20 fractions over 29 days the dose specification being in agreement with the recommendations in the ICRU Report 29 (1978). The tumour regressed rapidly and was no longer demonstrable 11 and 12 weeks after conclusion of treatment. Four months after the last irradiation day a local recurrence was demonstrated at radiography.

When the oesophageal tumour was still in complete remission the patient developed multiple subcutaneous tumours all being of the same microscopic type as the oesophageal tumour as confirmed by means of fine needle aspiration biopsy (Fig 1 c d).

It was decided to irradiate the metastases. Since the oesophageal tumour had responded well initially the fractionation schedule used i.e. 40 Gy in 20 fractions with treatment 5 days a week was taken as a reference. Then other schedules were chosen giving the same radiation effect on normal connective tissue in terms of Cumulative Radiation Effect (CRE=13.4 KIRK et coll 1971). An attempt was made to analyse the corresponding responses with

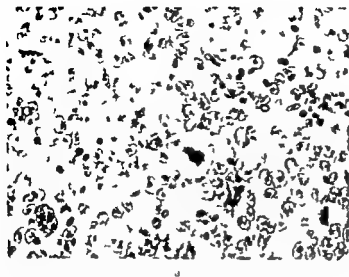
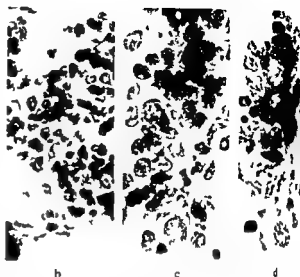


Fig. 1 a) Biopsy from oesophagus: undifferentiated malignant tumour with cellular pleomorphism and many mitoses. H & E $\times 360$. b) Imprint preparation from the same specimen. H & E



$\times 360$. c) and d) Fine needle biopsies from two metastases in the left upper arm. Cell clusters with some vague epithelial features. MGG $\times 360$.

respect to biologic effect even if the parameters of the schedules selected were restricted by the clinical situation. Five different fractionation schedules were used and altogether 7 metastatic tumours were irradiated (Fig. 3). These were also given with low LET radiation with the same RBE value. 11 MeV electrons from a Betatron or ^{60}Co gamma rays. The field sizes were chosen to encompass the palpable tumours with a margin of at least 2 cm. Chemotherapy was not administered. Each field irradiated with electrons was evaluated for any skin reaction at each fraction and during the subsequent follow-up. The reactions were divided into two groups: erythema (=1) and dry desquamation (=2).

As the metastases were subcutaneous, their sizes and shapes were easily evaluable by means of palpation and all tumours were well demarcated. The skin was freely movable and the distance from the tumours to the skin surface was measured to be 3 mm. All the tumours had the form of rotation ellipsoids, one being disc shaped and the others cigar shaped. The volume of a rotation ellipsoid is given by

$$V = \frac{\pi}{6} d_1 d_2 d$$

where d_1 and d_2 are the two independent axes of the ellipsoid (the longest and shortest diameters) and d_3 is the smaller axis for a cigar shape while it is the larger axis for a disc shape. At each irradiation and on several occasions after treatment the two axes d_1 and d_2 were measured with callipers independent

ly by three examiners and the tumour volume calculated according to the formula mentioned.

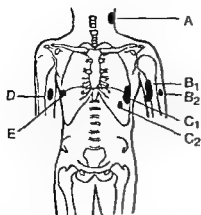
The location and initial size of the different tumours as well as data on the absorbed dose and fractionation are given in Fig. 2.

One additional tumour was not irradiated but followed for 57 days and then extirpated. This tumour had the same morphology as the other tumours.

Results and Discussion

In Fig. 3 the increase in accumulated absorbed dose with time is given for the 5 fractionation schedules together with different kinds of skin reactions both when first noticed and when most marked. After conclusion of treatment all observed skin reactions subsided. Skin reactions occurred only with the two schedules having the largest number of fractions, where is no reaction at all occurred with those having few fractions, even though the CRE value was the same for all the schedules. However, as TURSSON & NOTT (1975) stated, equal CRE values during the radiation treatment are not necessarily related to identical skin reactions as they are delayed. It is therefore difficult to compare skin reactions during the period of treatment itself to assess the results at the last occasion of treatment.

The tumour followed for 57 days progressed steadily before surgical excision and increased in size by approximately a factor of 11 from 0.7



Tumour	Volume (cm ³)	Absorbed dose (Gy)		No of fractions	No of days
		Accumulated	At each fraction		
A	3.68	40	7.00	70	30
B1	8.66	30	3.34	9	11
B2	0.64				
C1	4.63	29	5.80	5	30
C2	1.03				
D	1.64	17.80	8.90	7	3
E	0.65	15.40	2.95	17	70

Site and volume at start of treatment of the different subcutaneous tumours and data on target absorbed dose and fractionation. Tumours B and E were treated with ⁶⁰Co, the other tumours with electrons (11 MeV) from a Betatron.

to 4.18 cm³). From the two observations made, tumour volume doubling time T_d may be calculated assuming exponential tumour growth to ± 5 days.

The size of the different tumours as measured before and after the treatment period appears in Fig. 4. The uncertainties in all the measurements of the linear dimensions of the metastatic tumours are estimated to ± 1.5 mm. This gives the following approximate uncertainties in the volume calculations taken at three representative levels: (5.0 ± 1.0) , (0.50 ± 0.25) cm³ and (0.050 ± 0.045) cm³.

All metastases regressed completely and did not recur during a follow up time of 8 months. Therefore it is not possible to state if the absorbed dose is in excess of the necessary one to achieve cure. On the other hand, the primary oesophageal tumour recurred after 4 months.

It appears from Fig. 4 that no decrease of the tumours occurred during the first week of irradiation, rather an increase, in particular when large doses were given at each fraction. It is reasonable to attribute this increase to an initial oedema, even

if killed tumour cells are cells incapable of dividing and may still add to the tumour volume. Moreover, it cannot be ruled out that the increased tumour volume might reflect an accelerated growth rate of the surviving cells (HILL & BASERGA 1975).

In fractionated radiation several factors determine if all tumour cells will be destroyed or not.

Repair of sublethal injury is usually considered to take place within hours and at least before the next fraction in daily irradiation schedules. The size of the absorbed dose necessary to overcome the level of only sublethal injury was demonstrated by ELKIND & SUTTON (1960) as the initial shoulder of the cell survival curve in *in vitro* setups. The cell survival curve, which is usually obtained by plotting the proportion of cells in a population that survive to divide against the absorbed dose, was often assumed to have a zero initial slope (Fig. 5, curve A) as originally described by ELKIND & SUTTON. However, many data indicate that the initial slope may be non zero (Fig. 5, curve B, WAMBERSIE & DUTREIX 1974). The problem was extensively discussed by ELKIND (1976). Considerable evidence indicates a wide variation among different tumours with respect to the ability to repair sublethal injury. Some tumours, e.g. malignant lymphomas, are usually on clinical grounds considered to have relatively narrow shoulders, and then even small daily absorbed doses are useful (LANDBERG *et al.* 1973, FRIEDMAN 1975). Some tumours probably have broad shoulders. An example of this is malignant melanoma, where conventional fractionation with a daily absorbed dose of 2.0 Gy may be ineffective (BARANCO *et al.* 1971, THOMSON *et al.* 1975) and larger absorbed doses of the order of 4–8 Gy at each fraction are being explored (ROFSTAD *et al.* 1977).

Repopulation by tumour cells between successive fractions could be expected to present a clinical problem in tumours with a very high growth rate. This has been shown to be the case for some undifferentiated squamous cell carcinomas (FRIEDMAN) and is also observed in Burkitt's lymphoma, where conventional daily fractionation has proven insufficient, whereas a schedule with several fractions each day, superfractionation, has proven to be effective (NORIN *et al.* 1971). In most other malignant lymphomas, however, repopulation between fractions seems to be of little importance; the total treatment period may well be extended over a long period of time for one and the same total absorbed dose (KAPLAN 1966, LANDBERG *et al.*).

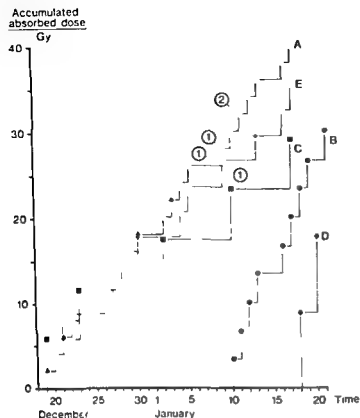


Fig. 3

Fig. 3 Increase in accumulated absorbed dose with different fractionation schedule and different kinds of skin reactions when first observed and most marked (only for sites irradiated with electrons): erythema (= 1) and dry desquamation (= 2). For explanation of symbols see Fig. 4.

Fig. 4 Size of the different tumours when measured at the beginning of, during and after radiation treatment. The uncertainties in the volume measurements are indicated in the right hand margin of the figure. For explanation of symbols see Fig. 3.

Fig. 5 Cell survival curves: A with initial slope zero and B with initial slope $n = \text{zero}$ (see review by ELKIND 1976).

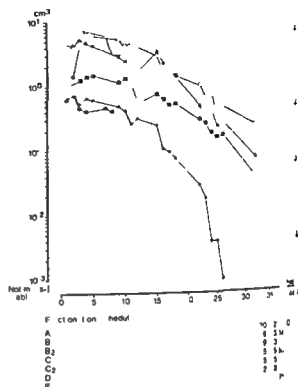


Fig. 4

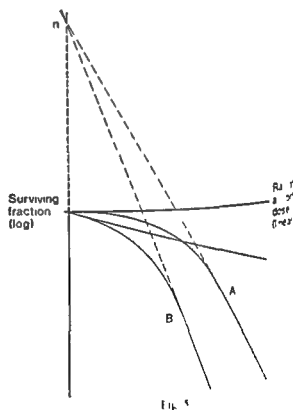


Fig. 5

Reassortment of cells into different phases of the cell cycle making surviving cells enter a period of increased proliferative activity has been described for human tumours. Thus TIRZ et coll. (1977)

showed that during radiation therapy of a tumour a significant increase in the thymidine incorporating cells occurs which was considered a increased proliferating activity of the tumour.

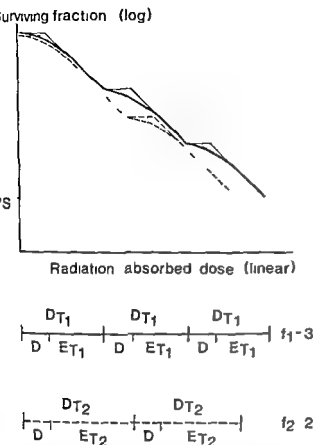


Fig. 6. Schematic presentation of two different radiation fractionation schedules giving the same final cell population survival fractions S . D_T absorbed dose at each fraction for schedule 1. D absorbed dose necessary to overcome the shoulder of the cell survival curve. E_T effective absorbed dose at each fraction ($= D_T - D$) for schedule 1. f number of fractions for schedule 1.

population. However this phenomenon seems to be of only limited importance during a fractionated treatment over a long time (FOWLER 1972).

Reoxygenation of hypoxic cells may be of importance causing a tumour to respond better (other factors unchanged) if the total treatment period is prolonged.

The size of the tumour is of importance because a larger tumour in comparison with a smaller one contains more tumour cells relatively more resting cells (G phase, STRAUS & MORAN 1977) and also may be assumed to contain higher hypoxic regions (THOMLINSON & GRAY 1955). That a clear correlation exists in the clinical situation between tumour size and dose necessary for local control has been shown by FLETCHER (1973) for different squamous cell carcinomas and adenocarcinomas.

The absolute preirradiation tumour size does not predict the dose needed, but how this size has been

reached seems to be of importance. Thus carcinomas of the oropharynx and maxillary antrum being shrunk before irradiation with an intra arterial infusion of methotrexate had not a better local control rate than far more advanced lesions with the same total absorbed dose (NERVI *et al.* 1978).

The response to irradiation may vary considerably between the metastases from the same primary tumour and probably even within the same metastasis. Such a variation in sensitivity by a factor of 2 or even more has been demonstrated by FRIEDMAN and TERZ *et al.* On the other hand STRAUS & MORAN demonstrated but little interlesional heterogeneity in cell cycle distribution in patients with solid tumours (long standing tumours). FOWLER remarked in his analysis of the results of SHUKOVSKY (1970) that they supported the simple cell survival curve theory, the variation in sensitivity being small.

An attempt was made to use the present observations to estimate the size of the shoulder of the cell survival curve, some reasonable assumptions having been made. It must be assumed that the different tumours did not differ with respect to biologic characteristics and that as a first approximation the effects of repopulation, reoxygenation and reoxygenation were the same in the different metastases. These assumptions are implicit in the calculation of the tumour volume doubling time T_d . Furthermore if the tumour volume is supposed to be proportional to the number of tumour cells, then the time needed for doubling the number of tumour cells is T_d as well. Apparently none of these assumptions can be substantiated by data which demonstrates a common difficulty in clinical radiation biology. Most biologic work on the effect of radiation has been performed with *in vitro* cell systems and animal tumours. This can never tell what happens *in vivo* in man and has even led to some misconceptions in the past (RUBIN & SCARANTINO 1978). Thus data from human beings are needed, although due consideration must be given the several assumptions made in the evaluation of the results as well as the clinical situation. As FRIEDMAN rightly stated:

Most published isoeffect curves of human cancer are based on impressment of data somewhat against their consent into a formula by the expedients of accepting data uncritically overlooking the enormity of the standard error and plotting data directly into logarithmic paper as to appear scientific.

The present observations indicate that the target absorbed doses given seem to have been at least

sufficient to eradicate the metastases but insufficient to cure the much larger primary lesion. The overall treatment times for the primary tumour and the four tumours A, C₁, C and E are the same, hence these metastases were chosen in the estimation of the shoulder in order to minimize the number of varying parameters. During the period, the relative changes in volume of these four tumours are approximately the same (Fig. 4). The curves do not only illustrate tumour cell killing, but also that other factors may be of importance, e.g. variation in tumour cell loss and stromal tissue reactions. For the present purpose it is assumed that such factors are of the same relative importance for the different sites, allowing for a comparison with respect to tumour cell killing if certain precautions are taken. Since the difference in initial size of the tumours A and C₁ is insignificant and it is reasonable to assume that the fractionation schedules used in the treatment of these two tumours finally result in the same surviving fractions, consequently also equal absolute numbers of tumour cells survive. The same situation applies for the two tumours C and E. The fractionation schedules for the tumours C₁ and C are identical and according to the assumptions made this means equal surviving fractions for these tumours. Thus the surviving fractions may be assumed to be equal for all four metastases, the variation in initial sizes being within a factor of 5.

The assumption that equal surviving fractions are reached using the three fractionation schedules A, C and E makes it possible to estimate the quasithreshold dose D , i.e. the width of the shoulder of the single cell survival curve. The quasithreshold dose D is the dose at which the extrapolated high dose straight part of the dose-response curve cuts the dose axis, when a presentation as in Fig. 5 is used. Thus the dose D is determined solely by the behaviour for large doses of the cell survival curve.

Two fractionation schedules resulting in the same surviving fraction S are compared in Fig. 6. The doses absorbed at each fraction are denoted D_{T1} and D_T and the number of fractions f_1 and f_2 respectively. The doses D_{T1} and D_T are presumed to be large enough to result in surviving fractions characterized by the straight part of the dose-response curve. This is the case in all the fractionation schedules analysed. The dose absorbed at each fraction D_T can be considered to be divided into two parts, where D is the dose needed to overcome the shoulder and F_T is the effective dose.

Table
Total effective absorbed dose on fraction f (Gy)

Tumour	Effective absorbed dose (Gy)	Initial volume of the tumour (cm ³)
A	25.20	1.68
B ₁	23.40	8.66
B ₂	23.40	0.64
C ₁	25.30	4.63
C	25.30	1.03
D	16.37	1.64
E	26.40	0.65
Primary tumour	25.20	65.0 (re-irradiated)

$$D_T = D + F_T$$

When the same surviving fraction S is reached by effective doses delivered by the two different treatment schedules are equal, that is

$$f_1 \times E_{T1} = f \times E_T$$

which means

$$(D_{T1} - D) \times f_1 = (D_T - D) \times f$$

The width D of the shoulder is then given by

$$D = \frac{D_{T1} \times f_1 - D_T \times f}{f_1 - f}$$

No conclusions about the specific shape of the shoulder can be made in such an analysis, in fact, if the condition on the size of the doses is satisfied, then the initial shape of the cell survival curve is arbitrary.

Comparison between the schedules for tumours A and C₁ results in the value $D_{T1} = 0.73$ Gy for the shoulder dose, tumours C and E give the value $D_{T1} = 0.93$ Gy and the schedules A and E give $D_{T1} = 0.58$ Gy. Thus the mean value $D_{T1} = 0.74$ Gy is an estimation of the shoulder of the cell survival curve based on equal surviving fractions in tumours C₁, C and E. Using the calculated value of the shoulder, total effective target doses can be determined. These effective doses are given in Table 1.

The primary tumour had a volume 20 to 100 times larger than the four tumours and was treated accordingly to tumour A. Even if the same surviving

of tumour cells was reached in the primary as the smaller tumours the absolute number of surviving cells was higher leading to the recurrence of the primary tumour. It is generally considered necessary to administer a higher dose to eradicate a larger tumour. For instance calculations according to COHEN's model (1971) give for a squamous cell carcinoma when a total dose of 60 Gy is given in 10 fractions over 41 days a 93 per cent chance of lethal effect on a tumour with a diameter of 1 cm while this chance is reduced to 75 per cent for a tumour with a diameter of 7 cm (350 times larger volume). A total dose of 67 Gy should be needed in order to reach the level of 93 per cent for this larger tumour: the number of fractions and the overall time being the same. FOWLER stated that a subclinical metastatic deposit 1 mm in diameter containing 10^6 cells can be eradicated by a dose which is two thirds as high as that required to eradicate a primary tumour of 10^9 cells.

FRIEDMAN stated that accelerated recovery from sublethal injury occurs in some squamous cell carcinomas when the destructive effect on the tumour is mild either because of a low daily dose (1.0 Gy) or because the tumour is very resistant.

The shoulder width arrived at seems reasonable from general clinical impressions.

The results of different well defined *in vitro* experiments may only be clinically useful if they are properly applied to the complex *in vivo* situation. What properly is can only be evaluated from observations in clinical work where on the other hand an investigation can be performed only if the end result is ethically acceptable. Therefore deductions from a clinical investigation should be viewed with the greatest caution.

SUMMARY

Subcutaneous metastases from an oesophageal carcinoma were irradiated using different schedules. The results have to be evaluated with greatest caution but indicate that with the same CRE value few fractions caused less skin reactions than several and the size of the shoulder of the cell survival curve was of the order of 0.7 Gy.

REFERENCES

- BARBARO S C, ROMSDALE M M and HUMPHREY M M. The radiation response of human malignant melanoma cells grown *in vitro*. *Cancer Res* 31 (1971) 830.
- COHEN L. A cell population kinetic model for fractionated radiation therapy. *Radiology* 101 (1971) 419.
- ELKIND M M. Fractionated dose radiotherapy and its relationship to cancer survival curve shapes. *Cancer Treat Rev* 3 (1976) 1.
- and SUTTON H. Radiation response of mammalian cells grown in culture. I. Repair of x ray damage in surviving chinese hamster cells. *Radiat Res* 13 (1960) 556.
- FLETCHER G H. Clinical dose-response curves of human malignant epithelial tumours. *Brit J Radiol* 46 (1973) 1.
- FOWLER J W. Current aspects of radiobiology as applied to radiotherapy. *Clin Radiol* 23 (1972) 257.
- FRIEDMAN M. Aspects of radiation biology and radiation pathology observed during the treatment of cancer in man. *Brit J Radiol* 48 (1975) 81.
- HILL M T and BASERGA R. The cell cycle and its significance for cancer treatment. *Cancer Treat Rev* 2 (1975) 159.
- ICRU. International Commission on Radiation Units and Measurements. Report 29. Dose specification for reporting external beam therapy with photons and electrons. Washington 1978.
- KAPLAN H S. Evidence for a tumoricidal dose level in the radiotherapy of Hodgkin's disease. *Cancer Res* 26 (1966) 1221.
- KIRK J, GRAY W M and WATSON E R. Cumulative radiation effect. Part I. Fractionated treatment regimes. *Clin Radiol* 22 (1971) 145.
- LANDBERG T, LIDEN K and FORSLO H. Split course radiation therapy of mediastinal Hodgkin's disease. TSD and CRE concepts. *Acta radiol Ther Phys Biol* 12 (1973) 33.
- NERVI C, ARCANGELI G, BADARACCO G, CORTESE M, MORELLI M and STARACE G. The relevance of tumour size and cell kinetics as predictors of radiation response in head and neck cancer. *Cancer* 41 (1978) 900.
- NORIN T, CLIFFORD P, EINHORN J, EINHORN V, JOHANSSON B, KLEIN G, ONYANGO J, DESCHRYVER A and WALSTAM B. Conventional and superfractionated radiation therapy in Burkitt's lymphoma. *Acta radiol Ther Phys Biol* 10 (1971) 545.
- ROFSTAD E K, BRUSTAD T and JOHANNESSEN J V. Effect of clinically relevant irradiation regimes on human malignant melanomas grown in athymic nude mice. *Acta radiol Ther Phys Biol* 16 (1977) 273.
- RUBIN P and SCARANTINO C W. The bone marrow organs. The critical structure in radiation-drug interaction. *Int J Radiat Oncol Biol Phys* 4 (1978) 3.
- SILKOVSKY L J. Dose time volume relationships in squamous cell carcinoma of the supraglottic larynx. *Amer J Roentgenol* 108 (1970) 27.
- STRAUS J M and MORAN R E. Cell cycle parameters in human solid tumors. *Cancer* 40 (1977) 1453.
- TERZ J J, LAWRENCE JR W and COX B. Analysis of the cycling and noncycling population of human solid tumors. *Cancer* 40 (1977) 1462.
- THOMLINSON R H and GRAY L H. The histological structure of some human lung cancers and the possible

- implications for radiotherapy. *Brit. J. Cancer* 9 (1955) 539.
- THOMSON L. F., SMITH A. R. and HUMPHREY R. M. The response of a human malignant melanoma cell line to high LET radiation. *Radiology* 117 (1975) 155.
- TURFSSON I. and NOTTER G. Skin reactions after different fractionation schedules giving the same cumulative radiation effect. *Acta radiol. Ther. Phys. Biol.* 14 (1975) 475.
- WAMBERSIE A. and DUTREIX J. Cellular repair after gamma irradiation determined for several gonorrhea on animals and patients. *Europ. J. Cancer* (1974) 235.

DEPTH DOSE DATA FOR 4 MeV LINEAR ACCELERATORS WITH LEAD OR URANIUM FIELD FLATTENERS

R. NAIR and D. E. WREDE

Precise dosimetry data is a prerequisite for an effective treatment planning system in radiation therapy. Per cent depth dose data obtained from two 4 MeV linear accelerators—one equipped with lead and another with depleted uranium as field flatteners—are compared with published values. This comparison clearly establishes that variations exist between depth doses which become very significant at depths greater than 10 cm of water.

Material and Method

Measurements were made on two Varian Clinac 4 linear accelerators: one an isocentrically mounted unit equipped with a lead field flattener and the other a stationary unit equipped with depleted uranium as the field flattener. A water phantom of size 66 cm × 60 cm × 66 cm and a Baldwin Farmer chamber of 0.6 cm³ volume connected to a Keithly 616 digital electrometer in conjunction with a model 6169 ion chamber interface were used for measurements. The calibration of the system was in accordance with the National Bureau of Standards, i.e. the Baldwin Farmer chamber was calibrated against ⁶⁰Co and a C_A of 0.94 was used. Each value reported is the average of 4 measurements.

Results

The measured central axis per cent depth dose values for 4 MV roentgen rays equipped with a lead

field flattener for a few selected depths are compared with values in the literature as indicated for a 10 cm × 10 cm field size at 80 cm SSD in Table 1. The per cent difference when the values are compared with those in Brit. J. Radiol. Suppl. No. 11 appear in Table 3. The per cent depth dose values flattened by a uranium field flattener are given in Table 2. The per cent difference in the values when compared with those of Brit. J. Radiol. Suppl. No. 11 is listed in Table 4.

Conclusion

The 4 MV roentgen rays flattened by a uranium field flattener have slightly higher penetrability and in addition give improved field features and dose homogeneity within the beam (NAIR & KARTHA 1978; NAIR et al. 1978) when compared to 4 MV roentgen rays using a lead field flattener. Values given in Brit. J. Radiol. Suppl. No. 11 cannot be taken for granted for 4 MV roentgen rays with different field flatteners since these values show that the error can be as much as 6 per cent compared with the present results and as much as 12 per cent compared with other published data when uranium field flatteners are used assuming depths greater than 10 cm. Thus direct measurement of individual beam parameters even in accelerators with supposedly similar characteristics must be emphasized.

- implications for radiotherapy. *Brit. J. Cancer* 9 (1955) 539.
- THOMSON L. F., SMITH A. R. and HUMPHREY R. M. The response of a human malignant melanoma cell line to high LET radiation. *Radiology* 117 (1975) 155.
- TURESSON I. and NOTTER G. Skin reactions after different fractionation schedules giving the same cumulative radiation effect. *Acta radiol. Ther. Phys. Biol.* 14 (1975) 475.
- WAMBERSIE A. and DUTRIEX J. Cellular repair after gamma irradiation determined for several parameters on animals and patients. *Europ. J. Cancer* (1974) 235.

Table 3

Percentage differences relative to Brit J Radiol depth dose values for 4 MV x roentgen axis
(lead field flattener) 10 cm x 10 cm at 80 cm SSD

Depth (cm)	Present values	Manufacturer's data	McCULLOUGH	PETERSON & GOLDEN	CASTRO & KOPENHAVER
2	-1.0	-1.9	-0.6	-0.4	+0.8
5	0.0	-0.5	+0.6	0.0	-1.1
10	+1.3	+1.7	+1.8	+0.5	-1.0
15	+0	+0.5	+1.1	+0.2	-1.9
20	+0.3	+0.6	+1.9	-0.3	-6.3
25	-0.4	+3.1	-	-0.9	-3.5
30	-0.6	+6.1	-	-1.8	-

Table 4

Percentage differences relative to Brit J Radiol depth dose values 4 MV x roentgen axis (uranium field flattener)

Depth (cm)	10 cm x 10 cm at 80 cm SSD				10 cm x 10 cm at 80 cm SSD			
	Present values	McCULLOUGH		Review Committee values	Present values	McCULLOUGH		Review Committee values
		Filter No 4	Filter No 6			Filter No 4	Filter No 6	
2	+0.3	-0.7	-	-	-0.1	-0.9	-	-
5	+1.2	+0.4	-0.1	+0.7	+0.8	+0.8	+0.5	-0.8
10	+1.8	+2.0	+1.5	+1.5	+3.3	+3.8	+3.0	-0.7
15	+3	+3.4	+2.7	+3.7	+5.3	+6.3	+4.9	+4.5
20	+4.4	+5.1	+5.1	+4.1	+6.2	+8.5	+7.3	-6.5
25	+3.5	-	+6.6	+5.3	+4.2	-	-8.7	+7.2
30	+3.0	-	+8.5	+8.5	+4.6	-	+9.1	+11.7

SUMMARY

Per cent depth dose data for a few selected depths for 4 MV x roentgen rays from Varian Clinac 4 linear accelerators equipped with lead or uranium field flatteners are presented and compared with published data. A difference by as much as 12 per cent was found.

REFERENCES

- British Journal of Radiology: Central axis depth dose data for use in radiotherapy (1972) Suppl No 11
 ASTRO V. G. and KOPENHAVER J. F. Some aspects of dosimetry with a 4 MV linear accelerator. Radiology 107 (1972) 691

Isodose Charts for Varian Clinac 4 supplied by the Manufacturer

McCULLOUGH E. C. Personal communication

NAIR R. and KARTH M. A study of dose distribution patterns from Cobalt 60 and Varian Clinac 4 beams. Amer J Roentgenol 130 (1978) 151

NAIR R., MENON N. S. K., BAUER C. and BAILEY F. Dosimetric aspects of 4 MV X rays from a linear accelerator equipped with a uranium field flattener. Appl Radiol 7 (1978) 81

PATERSON M. and GOLDEN R. Dosimetry of the Varian Clinac 4 Linear Accelerator. Radiology 103 (1972) 675
 Report of Uranium Filter Review Committee. Varian Radiation Division, Palo Alto, California, July 1975

DUAL PHOTON ABSORPTIOMETRY IN LUMBAR VERTEBRAE

Evaluation of the baseline error

B O ROOS, T H HANSSON and H SKÖLDBORN

Dual photon absorptiometry is a method of determining the bone mineral content in lumbar vertebrae *in vivo* (ROOS & SKÖLDBORN 1974, ROOS 1975). The method is based on the following principles:

Two nuclides ^{241}Am with a gamma energy of 59 keV and ^{137}Cs with a gamma energy of 662 keV are placed together under the examination couch. The patient lies on his back on the couch which travels in the transverse direction. The collimated gamma radiation beam which contains both photon energies is absorbed and partly scattered by the patient's skeleton and soft tissue. The transmitted fractions are detected with a NaI(Tl) detector placed in a frame over the patient. Stationary transmission measurements are performed at a series of points along a line transversally over the patient at the level of the third lumbar vertebra. The bone mineral mass m_b (in g/cm²) is calculated for each measurement point and plotted as a function of the position giving a bone profile curve. Integration over a subjective baseline between two end points gives the bone mineral content in g/cm.

By means of dual photon absorptiometry a two-phase system of bone mineral and lean soft tissue can be solved in a stationary measurement. The attenuation for adipose tissue differs from that of lean soft tissue causing a reduction of the registered bone mineral mass m_b at each individual measurement point (ROOS 1974). This systematic measurement error has also been observed and discussed in

connection with the more common single photon absorptiometry technique (WOOTEN *et al.* 1977, JUDY & VOGEL 1974). With both single and dual photon absorptiometry the scanning technique with integration of the bone profile curve is normally used to correct for a layer of fat which has a constant mass along the measurement path. The only influence of the fat in this case is a vertical shift of the bone profile curve.

Considerably more serious is the error caused by an inhomogeneous distribution of adipose tissue along the measurement path. A measurement path over L3 will generally pass through the caudal parts of the kidneys and thus the adipose capsules (Fig. 1). All measurement points on either side of the vertebra will be influenced by the adipose capsule. In the bone profile curve m_b in these areas will have a negative value. It is thus reasonable to assume that the end points of the baseline (E_1 and E_2 in Fig. 1) will also have negative values (assuming that the fat in the adipose capsules of the kidneys is the only inhomogeneously distributed fat). After integration the bone mineral content will be overestimated by an amount which is approximately equal to the area A_2 .

In order to test this hypothesis a series of measurements on the bone mineral content in the third lumbar vertebra was carried out in a number of cadavers using exactly the same technique as is

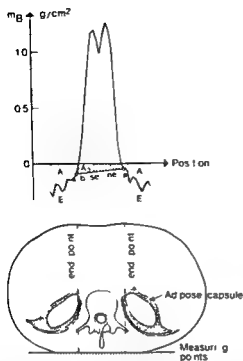


Fig. 1 Influence of inhomogeneous adipose tissue distribution along the measurement path. Negative values of bone mineral mass m_B due to influence of fat form the areas A_1 and A_2 . The integrated area between the end points E_1 and E_2 represents the bone mineral content which will be overestimated by the area A .

used *in vivo*. A segment of the lumbar spine comprising vertebrae L2–L4 was then removed and measured under controlled conditions in a water bath. The technique was the same as was previously used in determining the mineral content in vertebrae

Table

The age, sex and bone mineral content in vertebra L3 *in situ* (BMC_i) and *in vitro* (BMC_v) and the difference between BMC and BMC_i in 14 cadavers

No	Age	Sex	BMC	BMC _i	BMC – BMC _i
1	54	M	4.74	4.09	0.15
2	55	M	5.19	5.16	0.53
3	56	M	3.37	3.87	-0.45
4	59	M	3.78	3.47	0.31
5	59	M	4.87	4.54	0.33
6	60	M	3.71	3.81	-0.10
7	64	F	4.7	3.34	0.84
8	64	M	4.7	3.97	0.30
9	69	M	5.09	4.50	0.59
10	70	F	3.84	3.1	0.61
11	77	M	8.4	8.40	-0.17
12	74	F	4.56	4.40	0.46
13	74	M	5.79	5.17	0.12
14	75	M	4.50	3.61	0.89

in relation to compressive strength (Hägg 1979). The present report presents the results of measurements on lumbar vertebrae from cadavers and analyses the difference between *in vivo* and *in vitro* measurements in order to evaluate conclusions from biomechanical tests of compressive strength (HANSSON *et al.*

Material and Methods

The material comprised 14 cadavers (50–75 years). The sex, age and results of the measurements appear in the Table. The only form of exclusion was individuals who had malignancies or other diseases which might affect the skeleton. The most usual cause of death was myocardial infarction.

For the *in situ* measurements the cadaver was placed in the supine position on the examination couch and vertebra L3 located by fluoroscopy. Recordings were then made at 35 points along the measurement path transversely over L3. The measurement time was 0.7 min for each measurement. The distance between the measurement points was 10 mm. The recording for each measurement was designated m_B , the bone mineral mass in L3. This was plotted as a function of position giving a profile curve (Fig. 1). The bone mineral content (BMC) was calculated from the bone profile curve. The end points were selected such that the points lying closest to the profile of the vertebra which were projected free from the capsule. Normally the full width at half maximum of the curve may be considered to represent the breadth of the vertebra and the end points were selected 12 mm outside FWHM. The area between the end points is expressed by the following equation:

$$BMC = \left[\sum_{i=1}^n m_B^{(i)} - \frac{n-2}{2} (m_B^{(1)} + m_B^{(n)}) \right] \times 0.1$$

where the end points are numbered (1) and (n). $m_B^{(i)}$ is the bone mineral mass at a point between the end points. The bone mineral content *in situ* is designated BMC_i.

Vertebrae L2–L4 were removed in one piece for autopsy and *in vitro* measurements then performed over L3 using the same method as was used in the previous *in vitro* experiments on the compressive strength of vertebrae in relation to the bone mineral content (HANSSON *et al.* 1979), i.e. with the

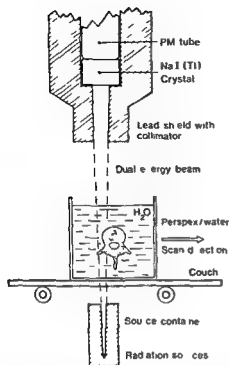


Fig. 2 Experimental set up for in vitro measurement of lumbar vertebrae. The preparation (L7-L4) is fixed in a water bath on the scanning examination couch and intermittent scanning is performed in the same way as in vivo and in situ measurement.

fixed in a water bath as shown in Fig. 2. The measurements, profile drawings, end point determinations and calculations of the mineral contents were made in the same way as in the in situ measurements. Measurement in vitro was considered to represent the bone mineral content of the vertebra without interference from adipose tissue. This value was designated BMC.

The difference between BMC₁ and BMC₂ was considered to represent the systematic error caused by erroneous positioning of the baseline. This difference was calculated for each vertebra and is given in the Table.

Examples of bone profile curves in situ and in vitro appear in Fig. 3.

Results

In the Table the BMC is given in g/cm. The subjects are listed in order of age. The Table shows BMC₁, BMC₂ and the difference between BMC₁ and BMC₂. It appears that the BMC difference varies from -0.45 (preparation No. 3) to 0.89 (preparation No. 14). The values of the BMC difference are fairly

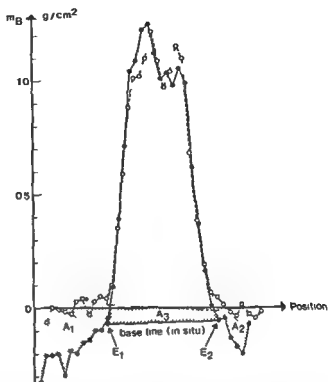


Fig. 3 Bone profile curves for measurement in situ (solid line) and in vitro (dashed line) for subject No. 14 in the Table. The two profile curves have been adjusted in the vertical direction in relation to the full width at half maximum of the curves. The adipose effect designated A₁ in Fig. 1 was in this case 0.53 g/cm.

evenly distributed between these extreme values. It can thus not be assumed that the BMC difference is normally distributed and a non parametric method of statistical analysis and evaluation of significance must be chosen. The sign test was chosen. The BMC difference in the Table has a median value of 0.32. According to the sign test the median value is significantly greater than zero with the confidence interval (0.12-0.59) at the confidence level 94.4 per cent.

The median value for BMC₁ is 4.39 (mean values for preparations Nos 8 and 14).

The median value for BMC₂ was 3.90 (mean value of BMC for preparations Nos 3 and 8).

Discussion

The results thus confirm the hypothesis that in situ measurement (and thus also in vivo measurement) gives a significantly higher figure than is obtained on measurement of the same vertebra in vitro (confidence level 94.4%). This seems to be due to the fact that the end points of the baseline are

systematically positioned too low during in vivo measurement due to interference by adipose tissue on either side of the vertebra.

A high correlation ($r=0.86$) between the bone mineral content of lumbar vertebrae and their ultimate compressive strength has previously been found in vitro (HANSSON *et coll.*). Comparison between biomechanical properties of vertebrae in vivo and in vitro implies important potential sources of error. The present results suggest that the bone mineral values in in vivo measurements systematically overestimate the mechanical strength of the vertebrae: the error being of the order to 250 N (HANSSON *et coll.*).

The differences between in vivo and in vitro measurements may have been influenced by the composition of the patient material. Most of the vertebrae examined belonged to men (11/14). The amount of fat around the kidneys in men is probably greater than in individuals with osteoporosis since short slender elderly women dominate among the latter (SAYVILLE & NILSSON 1966). The difference between the bone mineral content in vivo and the in vitro value in these individuals is thus probably smaller than was found in the present series (0.32 g/cm).

Based on the attenuation coefficients for adipose tissue, water and hydroxyapatite (ROOS 1974), the median difference of 0.32 g/cm may be calculated to correspond to a fat mass of just over 1.4 g/cm at the end points of the baseline.

SUMMARY

In connection with determination of the bone mineral content in the third lumbar vertebra by dual photon ab-

sorptiometry the fat in the adipose capsules of the kidneys is assumed to cause erroneous position of baseline, leading to overestimation of the bone content. The bone mineral content in L3 was measured in situ (BMC_i) and in vitro (BMC_v) in 14 cadavers. The difference between BMC_i and BMC_v was significantly greater than zero: the median value being 0.32 g/cm at the confidence level of 94.4 per cent. It is concluded that the relation between bone mineral content and compressive strength in vitro: the in vivo strength is overestimated about 250 N.

ACKNOWLEDGEMENT

This investigation was supported by grants from the Gothenburg Medical Society and from the Swedish Medical Research Council.

REFERENCES

- HANSSON T, ROOS B and NACHEMSON A. The bone mineral content and ultimate compressive strength of lumbar vertebrae. *Spine* 4 (1979) 79.
- JUDY P F and VOGEL J M. A method to estimate the error caused by adipose tissue in bone absorptiometry. In: *Proceedings of the symposium on bone mineral determinations*. Stockholm: Studsvik, 1974.
- ROOS B O. Dual photon absorptiometry in lumbar vertebrae. Thesis: University of Gothenburg, 1974.
- Dual photon absorptiometry in lumbar vertebrae. Precision and reproducibility. *Acta radiol Ther Phys Biol* 14 (1975) 291.
- and SKÖLDBORN H. Dual photon absorptiometry in lumbar vertebrae. I. Theory and method. *Acta radiol Ther Phys Biol* 13 (1974) 266.
- SAYVILLE P D and NILSSON B I. Height and weight, symptomatic postmenopausal osteoporosis. *Acta orthop* 45 (1966) 49.
- WOOTEN W W, JUDY P F and GREENFIELD M L. Analysis of the effects of adipose tissue on the absorptiometric measurement of bone mineral mass. *Invest Radiol* 8 (1973) 84.

MICRODOSIMETRY

I Use of secondary electron emission

B J FORSBERG and T E BURLIN

Microdosimetry is concerned with the stochastic nature of the deposition of energy by ionising radiation. Since the original work of ROSSI (1949) the subject advanced rapidly at both the theoretic and experimental level. The ultimate aim has been to determine the statistical nature of the energy deposition in biologic material, particularly human tissue. The experimental approaches have been dominated by measurements of ionisation produced in gases, which of the theory and its associated input data derives from information based on the gaseous state of human tissue, which consists largely of water. It would best be modelled using the liquid state, but present techniques and knowledge do not make this possible. The solid state being a condensed phase of similar density is in principle at least as good a model of the liquid state as gases and possibly better. It has been suggested (BURLIN 1974) that secondary electron emission is a phenomenon which could lend itself to microdosimetry. This report presents an exploration of this suggestion.

Secondary electron emission as an alternative technique

When a medium is irradiated by roentgen rays or electrons, a degradation spectrum of electrons is established within the medium. This spectrum covers an energy range from zero up to some maximum energy. Selecting the bottom of the conduction band as a suitable reference level in a con-

ducting material, the general shape of the energy spectrum is represented in Fig. 1 (McCONNELL et al. 1966). Now consider what happens when this electron spectrum passes through the interface between the material and a vacuum. The electron spectrum may be conveniently divided into three energy regions (Fig. 1a). First there is an energy band A where the electrons will not have sufficient energy to cross the potential barrier at the surface. Secondly, an energy band B exists where some of the electrons will penetrate the potential barrier and escape from the surface with an energy of 0–50 eV. These only will be referred to as secondary electrons in the following. The cut-off at 50 eV is of course arbitrary but has been widely accepted in the literature of secondary electron emission. Thirdly, there is an energy band C of fast electrons which will penetrate the potential barrier and emerge from the surface virtually unmodified. In secondary electron literature this part of the degradation spectrum is often referred to as rediffused primary electrons and elastically reflected primary electrons. The potential barrier modifies the energy spectrum of the secondary electrons S so that outside the medium their energy spectrum is as shown by the broken line in Fig. 1. The secondary electron spectrum has a marked peak at 1 to 3 eV outside the medium.

The secondary electrons have a very limited range in a solid due to their interaction with the electrons

Electron flux

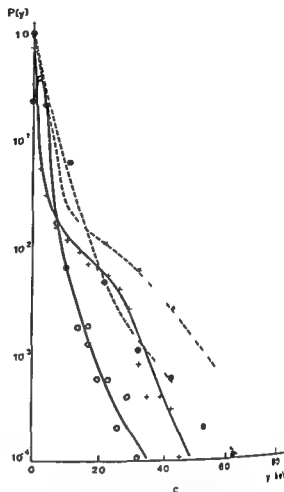
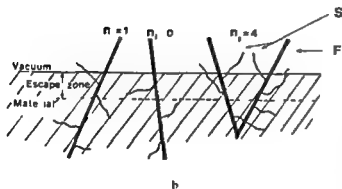
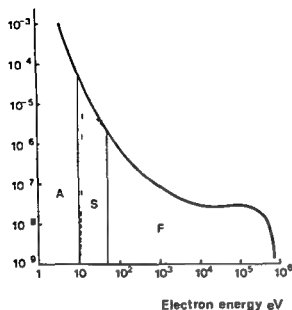


Fig. 1 a) Degradation electron energy spectrum as radiated medium. Broken line represents the electron spectrum external to the medium. b) Generation of secondary electrons S by fast electrons F. n is number of secondary electrons liberated by the 3 fast electrons shown. c) Probability distribution of lineal energy from the emission of secondary electrons reflected from thick targets of carbon and lithium. $k=1$ carbon (\bullet), $k=16$ carbon (\odot), $k=16$ Li (\star), $k=16$ Li ($+$).

and phonons so that those secondary electrons emitted originate in a very thin surface layer from which secondary electrons are able to escape. This layer is known as the escape zone. Beyond this depth no secondary electrons are defined will escape. Its thickness is a characteristic of the particular material but is independent of the energy of the primary radiation. The depth of the escape zone is up to 10 nm for metals (DJATLOWITSKAJA 1948) and about 50 nm or more for insulators (NAKHODKIN & ROMANOWSKY 1958; BRONSHTEIN & SEGAL 1960; BRONSHTEIN & FRAIMIN 1961). Secondary electrons emerging from a surface therefore reflect the energy deposition within volumes of linear dimensions of approximately 10 nm. Thus it is possible to analyse energy deposition in volumes very much

smaller than those which can be simulated by conventional proportional counter techniques. These are limited to volumes of linear dimensions of approximately 0.5 μm and above.

Energy deposition and secondary electron emission are however a stochastic process illustrated diagrammatically in Fig. 1b by three fast electrons. Each fast electron passing through the escape zone (in a few cases more than once) constitutes an event in which different amounts of energy are deposited into the escape zone. The energy deposited produces secondary electrons in the escape zone some of which are emitted from the surface. The fluence (there is no stochastic analogue of fluence defined so the term local has been introduced) of secondary electrons which may be measured

ts the fluctuation of energy depositions within escape zone

Clear analogies exist between the measurement of ionisation in a gas and the measurement of secondary electrons emitted from the surface of a solid

(1) The sensitivity volumes (the volume from which the ionisation is collected in the case of a gaseous detector and the escape zone in the case of a solid surface emitting secondary electrons) may be reversed by a complete charged particle spectrum measurement originating at large distances from the sensitivity volume

(2) The number of electrons liberated by a primary particle of fixed energy and direction in the sensitive volume is subject to statistical fluctuations. If the primary particle is an electron the number of electrons liberated in the sensitive volume in a normal proportional counter or from the escape zone of a metal or insulator is small, often zero. The number of electrons liberated in a single event (associated with one primary particle) in a gas or solid is therefore not related to the energy deposition from that event in the sensitive volume by a simple constant of proportionality

(3) Nevertheless it is possible to identify a constant of proportionality which, when averaged over a very large number of events, relates the electric charge released to the energy deposition in the sensitive volume. In the case of the gas, the constant of proportionality is the average energy expended in the gas per ion formed, W . This average embraces all mechanisms of energy loss other than ionisation, such as excitation by the primary particle. In the case of the solid, the constant of proportionality is the average energy expended in the escape zone per secondary electron emitted, W_{se} . This average embraces mechanisms of energy loss by the primary particle within the solid, other than secondary electron emission, including various forms of excitation.

(4) The stochastic quantity lineal energy, y , is the quotient of the energy imparted to the matter in a volume during an energy deposition event by the mean chord length in the volume. In the case of the gas, the ion pairs produced are measured and multiplied by a constant W to determine the lineal energy spectrum. In the case of the solid, the secondary electrons released from the surface may be measured and multiplied by a constant W_{se} to determine the lineal energy spectrum.

Some differences exist also between the two

techniques. First, the escape zone of a solid is effectively an infinite thin slab rather than a finite domain and may thus simulate a membrane rather better than a gas volume. Secondly, unless a plate of identical material is placed close to the surface emitting secondary electrons, electronic equilibrium is unlikely to exist as is often the case with proportional counters. This is not an objection in principle to the secondary electron emission technique, but the spectrum of fast charged particles crossing the interface would have to be carefully considered in any experiment.

Although not undertaken in a microdosimetry context, some experiments on the stochastics of secondary electron emission have been performed and the techniques would appear to be no more formidable than those currently used in microdosimetry. For example, McDONALD *et al.* (1973) operated with a vacuum of 10^{-8} torr, which is a relatively easy vacuum to obtain with modern techniques. The secondary electrons emitted from a surface were accelerated through 45 kV to a lithium drifted silicon detector pulse amplifier with a resolution of 3.1 keV. The number of secondary electrons per event was recorded by a multi-channel analyser and this is the basic data of microdosimetry from which y spectra and mean values can be obtained. They also undertook Monte Carlo calculations of energy deposited within an escape zone and the associated secondary electron emission, which agreed well with their measurements.

Stochastic aspects of secondary electron emission

When a material is irradiated secondary electrons will be emitted from the surface. The literature describing various secondary electron emission phenomena is extensive but work concerning the stochastic aspects of the emitted secondaries is sparse. Some of the work reported is presented in Table 1. They all describe measurements or calculations of the probability distributions, $P(n)$, of the number of secondary electrons emerging per incident fast electron from different surfaces.

There is some concentration in the choice of materials and energies of the primary particles examined. This is because the materials investigated (alkali halides) are all common as sensitive layers in dynodes in photomultipliers and in the energy range 1 to 8 keV maximum yield of the secondary electrons occurs for the thickness commonly examined.

Table I

Investigations concerning the stochastic of secondary electron emission

Reference	Material	Radiation	Calculation	Measurement
DELANEY & WATSON (1966)	Al	5.3 MeV α particles	No	Yes
DILLTZ et coll. (1966)	KCl KCl + AlO	8-9 keV electrons 25 keV ions	Yes	Yes
PRESCOTT (1966)	General	Electrons	Yes	No
LLACER & GARWIN (1969b)	CsI KCl LiF	^{90}Sr - ^{90}Y β rays 9 keV electrons	Yes	Yes
WILCOCK & MILLER (1969)	CsI KCl	≤ 10 keV electrons	No	Yes
GRILPNER (1971)	Al Cu etc	Positive ions	No	Yes
MCDONALD et coll. (1973)	CsI KCl NaCl LiF	5.9 keV roentgen rays	Yes	Yes
CAFOLLA et coll. (1975)	CsI KCl LiF	≤ 5 keV electrons	Yes	Yes

One of the materials (LiF) can be regarded as near tissue equivalent as far as its absorption characteristics for directly and indirectly ionizing particles are concerned. LLACER & GARWIN (1969b) have made measurements of the secondary electron emission from different materials irradiated with β rays (^{90}Sr - ^{90}Y) and calculations with 1 MeV and 7 to 9 keV electrons as primary particles. They employed a dielectric theory to account for the energy losses and the electron-phonon interaction to determine the probability that a second electron will reach the surface and escape. They suggest the following formula for this escape probability

$$P(e_0, x) = P_0(e_0, 0) \exp(-x/L)$$

where $P(e_0, x)$ is the probability that an electron generated with an energy e_0 above the conduction band at depth x will reach the surface and escape and L is a characteristic escape length. A more accurate formula for the escape probability was obtained by weighting the sum of exponentials over the energy spectrum of the secondaries.

MCDONALD et coll. (1971-1973) have developed a Monte Carlo program with which they make calculations of the electron transport through different materials. The probability of escape is determined in the same way as by LLACER & GARWIN. The probability $P(e_0, 0)$ of an electron of energy e_0 situated at the surface ($x = 0$) escaping was equal to 0.75 for all their calculations. Therefore they had only to choose the overall energy loss corresponding to the

production of one internal secondary electron and the characteristic escape length L for each material. Their computations were in good agreement with measurements made using the 5.9 keV roentgen rays from ^{55}Fe .

While most authors have calculated the second electrons emerging from the exit surface of a material, CAFOLLA et coll. (1975) have made calculations of the secondary electrons emerging from the entrance surface. Experimental results employing low energy electrons concur with these calculations.

Theoretical exploration of technique

The calculations are based on a Monte Carlo program described in detail by MCDONALD et coll. (1971-1973) (see also GREEN 1963 and OSTROM & HOV 1967).

The first electron tracks are divided into segments. Each segment is terminated by an elastic Rutherford scattering from the nucleus which is governed by the equation

$$\frac{d\sigma}{d\Omega} = \left(\frac{Ze^2}{4E} \right)^2 \left(\sin^2 \frac{\theta}{2} + \frac{\phi^2}{4} \right)^{-1}$$

where $\frac{d\sigma}{d\Omega}$ is the differential cross section per solid angle

Z is the atomic number of the absorbing material

e is the electronic charge

E is the instantaneous energy of the electron

θ is the angle of deflection

ψ is a correction factor to the simple Rutherford formula to take account of the screening effect of the orbital electrons when the incident electron passes the nucleus at a large distance

Three conditions of randomness are introduced each elastic scattering by the following three Monte Carlo equations

$$\frac{\varphi}{2\pi} = \gamma \quad (2)$$

$$\frac{\int_0^1 (\frac{d\sigma}{d\Omega}) \sin\eta \, d\eta}{\int_0^\pi (\frac{d\sigma}{d\Omega}) \sin\eta \, d\eta} = \beta \quad (3)$$

$$\exp(-x/\lambda) = \alpha \quad (4)$$

where α , β and γ are random variables uniformly distributed in the interval (0, 1). Eqs 2 and 3 give the direction of the new segment of the track with respect to the previous one. Eq 2 indicates that the choice of φ the other polar co-ordinate (with θ) defining direction is arbitrary. Eq 3 allows a random choice for θ which accords nonetheless with the general angular distribution of eq 1. Eq 4 allows the selection of a random distance x to the next collision (i.e. the length of the next segment) λ being the mean path length.

The energy loss in a segment of electron track is determined by the Bethe formula

$$-\frac{dE}{dx} = \frac{4\pi e^4 N Z}{mv} \ln\left(\frac{mv}{2I}\right) \sqrt{\frac{1}{2}} \quad (5)$$

This energy loss in the segment is attributed equally to distant and near collisions. The half of the energy attributed to distant collisions produces excitation of the atoms concerned and is considered to be deposited in that segment of the track. The half of the energy attributed to near collisions produces ionisation and results in collisions occurring randomly once in every k segments. McDONALD et al (1971) chose the value of k by obtaining a good fit to the experimental energy spread of electrons emerging from an aluminium foil. Their value of $k=16$ is also employed in the present calculations. Thus the energy attributed to near collisions may be deposited in another segment of track together with energy from near collisions from k segments. There are a very large number of distant collisions with very small energy loss per collision. The number of

near collisions are few in number with a large energy loss per collision though the energy loss per collision is still small compared with the energy of the fast electron. Thus secondary electrons are produced only in segments of tracks where near collisions occur. The number of secondary electrons produced is determined by dividing the energy lost in the near collision by the average energy required to produce a secondary electron in the material.

The interaction of these secondary electrons which have very low energy is predominantly with the phonons. As already noted the probability that a secondary electron generated with energy e_0 at a depth x will reach the surface and escape is $P_0(e_0, 0) \exp(-x/L)$. Strictly both $P_0(e_0, 0)$ and L are functions of initial secondary electron energy and should be appropriately weighted over the energy spectrum of the secondaries. However adapting average values for each material has proved completely satisfactory (McDONALD et al 1973).

The probability $P(n)$ that n secondary electrons will emerge per primary electron was calculated with $k=1$ and $k=16$. When this is multiplied by the average energy expended in the escape zone per secondary electron emitted the probability distribution of the number of events in event size $P(y)$ is obtained. Thus the measurement of the secondary electrons illustrated in Fig 1b can provide the probability distribution of lineal energy deposited in the escape zone as is illustrated in Fig 1c. Hence the frequency mean and the dose mean of lineal energy may be obtained. A continuous energy loss model for electrons is represented when $k=1$. The probability distribution will then only be caused by the differences in the track lengths (sum of track segments) of the electron within the escape zone and the relative variance can be shown to be proportional to $\ln(\text{constant}/L)$. Thus as shown in Fig 1c the relative variance for lithium fluoride which has a thick escape zone is less than for carbon which has a thinner escape zone. A discontinuous energy loss model for electrons is represented when $k=16$ and again the relative variance due to energy loss straggling is greater for carbon the material with the thinner escape zone.

Following McDONALD in these calculations $k=16$ was employed. Calculations of the probability distributions were carried out for monoenergetic electrons with initial energy between 6 and 30 keV directed normally towards infinite thick targets and the distributions were determined at the entry side

Table 2

Input parameters to the calculations. I is the average excitation ionisation potential, Z is the atomic number, L is a characteristic escape length, ϵ is the energy loss corresponding to the production of one internal secondary electron, $P(\epsilon_0, 0)$ is the probability that an electron having an energy ϵ above the conduction band at zero depth will escape.

Material	I (eV)	Z	L (nm)	ϵ (eV)	$P(\epsilon_0, 0)^a$	W_{ei} (eV/electron)
Carbon	78	6	1.3^d	11	0.22	61^f
Lithium fluoride	87.2	8.2	8.0^c	36	0.80	66
Aluminium	164^b	13	1.3^d	13	0.60	35^f

ICRU Report 16 (1970)

BISCHIEL (1968)

LIACER & GARWIN (1969a)

BRONSHTEIN & SEGAL (1960)

FITTING et al. (1976)

KANTER (1961)

Data for primary electron energies below 10 keV

Best fit to experimental results of KANTER (1961)

Similar calculations were also made for electrons up to 60 keV directed towards thin films of different thickness and the distributions were determined at the exit side.

Choice of parameters The different parameters used in the calculations are listed in Table 2. A comparison of these calculations with experimental measurements is presented in Fig. 2 and is taken to indicate that the choice of input parameters is reasonable. The sources of these input parameters are described in the following.

The overall energy loss corresponding to the production of one internal secondary electron ϵ of 36 eV for LiF was taken from LIACER & GARWIN (1969a) though it is noted that McDONALD et al. (1973) obtained a best fit to their experimental results using 48 eV. Below a primary electron energy of 10 keV the value for aluminium was derived from FITTING et al. (1976) who calculated the secondary yield inside a target per primary particle and knowing the energy dissipated in the target derived ϵ values for different materials. An interpolation from their data was made for carbon. For higher primary energies ϵ was obtained by finding the best fit between calculation and measured yield values of REIMER & DRIESCHER (1977) for aluminium at higher energies, employing the values of $P(\epsilon_0, 0)$ in Table 2. An interpolation was made for ϵ for carbon at higher primary energies. For silicon and germanium the average energy corresponding to an electron hole pair is in the range 3–4 eV; this may be compared with ϵ for the materials listed in Table 2.

The choice of the value of ϵ and $P(\epsilon_0, 0)$ is interdependent so that increasing the $P(\epsilon_0, 0)$ by 10 per cent will have approximately the same effect as decreasing the ϵ value by 10 per cent. Therefore it is always possible by means of comparison with experimental data to minimise possible errors in $P(\epsilon_0, 0)$ and ϵ . In fact $P(\epsilon_0, 0)$ was determined by finding the best fit with experimental data of KANTER (1961, Fig. 2). Values of 0.60 and 0.77 for Al and C respectively were obtained.

An alternative approach to estimating $P(\epsilon, 0)$ which provides a check on these values is to note that $P(\epsilon_0, 0)$ is closely related to the expression (BURLIN 1974)

$$P_0(E) = 1 - \left[\frac{F_{mi}}{E} \right]^2$$

where $P_0(E)$ is the probability that an electron of energy E will escape from the surface and F_{mi} is equal to the electron affinity of the crystal for insulators and equal to the sum of the photoelectron work function and Fermi energy for metals. For alkali halides the electron affinity is a few tenths of one eV and taking from LIACER & GARWIN (1969a) a value of 3 eV for the mean energy of the secondary electrons, $P(E)$ is derived to be 0.8. If the electron energy spectrum established within the material is represented with respect to the Fermi level by an average energy of 8 eV, a figure of the $P(E)$ for Al could be derived. With a photoelectron work function for C of 4.8 eV and for Al of 4.3 eV, values for $P(E)$ of about 0.4 and about 0.2

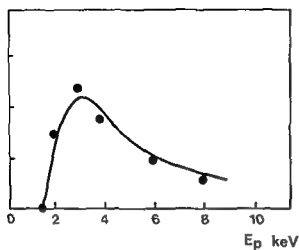


Fig. 1a

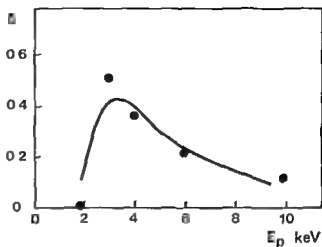


Fig. 2b

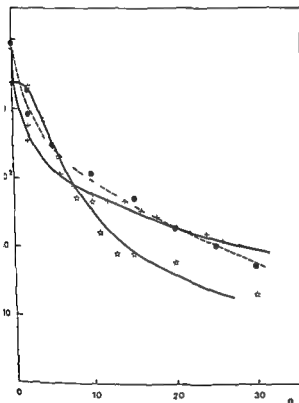


Fig. 3

Fig. 2 Comparison with experimental yield curves obtained from the exit side of foils by KANTER (1961): a) 17.5 µg/cm² thick Al foil b) 71.5 µg/cm² thick C foil. Experimental results (—) Calculated (●)

Fig. 3 Probability distributions for emission of secondary electrons from the exit side of a lithium fluoride foil bombarded with 7 keV electrons. Experiment by LLACER & GARWIN (1969b) (●) calculated $k=1$ (○) calculated $k=16$ (+)

derived for Al and C respectively. These estimates are in reasonable agreement with the values obtained by fitting the data of KANTER.

A common method to determine the characteristic escape length L is to equate it to one third of the escape zone. In LiF LLACER & GARWIN (1969) considered an electron energy of 8 eV to have a maximum escape depth of 25 nm which corresponds to a value of 8 nm. McDONALD et al. (1973) got a

best fit with their experiments using 8 nm, providing some confirmation of this value. According to BRONSTEIN & SEGAL the maximum escape depth is 10 to 20 atomic layers for metals, corresponding to a characteristic escape length of 1 to 2 nm.

An examination indicates that the calculations were not very sensitive to the value of L or ϵ . Thus L was increased by a factor of two, the dose mean of the lineal energy y_D increased with 13 per cent. If ϵ

was increased from 38 eV to 58 eV for 6 keV electrons incident on LiF the dose mean of the lineal energy y_D decreased 10 per cent though it should be noted that increasing L by a factor of two will not result in any good fit.

The average energy expended in the escape zone per secondary electron emitted W_{st} is required to derive the energy deposition. This quantity was taken for C and Al from KANTER and derived for LiF from LIACER & GARWIN (1969a). These values are strongly dependent on the cleanness of the surfaces through which the electrons escape and should be regarded as approximate but nevertheless providing a picture of the energy deposited in the sensitive volume.

Comparison with yield curves are an inadequate demonstration when the objective of the calculation is the complete $P(n)$ distribution. Therefore a calculation for 7 keV electrons directed on a thin film were compared with the experimental results of LIACER & GARWIN (1969b). Fig. 3 clearly demonstrates the effect of energy straggling. Using $k=16$ and the parameters listed in Table 2 the agreement between the curves present in Fig. 2c is satisfactory.

Results and Discussion

The objective of this report is to illustrate the potential of secondary electron emission as a technique for microdosimetry. An extension of the use of proportional counters to volumes with dimensions equivalent to less than $0.2 \mu\text{m}$ in tissue presents formidable difficulties and a probable increased uncertainty in the measured quantities. In contrast data are presented in Figs 4 and 5 for the lineal energy probability distributions for volumes with linear dimensions in the nm region. However it should be noted that the definition of lineal energy involves the mean chord length \bar{l} which for a thin slab under isotropic irradiation conditions is equal to twice the thickness ($\bar{l} = 2$ times the characteristic escape length (BIRKHOF et al. 1970)). Taking the characteristic escape length as one third of the escape zone resulted in mean chord lengths in unit density material of 6.7 and 42 nm for carbon, aluminium and lithium fluoride respectively. Since no isotropic conditions exist the values mentioned should be considered as upper limits. Fig. 4 presents data relevant to secondary electrons emitted from

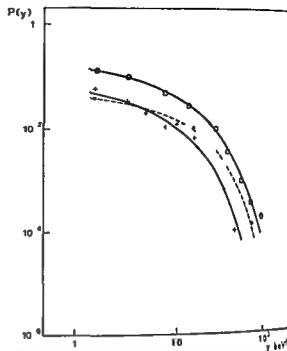


Fig. 4. Probability distribution of lineal energy per unit path length based on secondary electrons emission from thick targets of carbon, aluminium and lithium fluoride bombarded with 6 keV electrons. Points show the fluctuations of the calculated results. For C and Al the points in the low lineal energy interval are interpolated values between $P(0)$ (the probability of no secondary electrons emitted) and the probability of exactly one secondary electron emitted. For C (O) $P(0)=0.94$ for Al (X) $P(0)=0.94$ for LiF (+) $P(0)=0.77$.

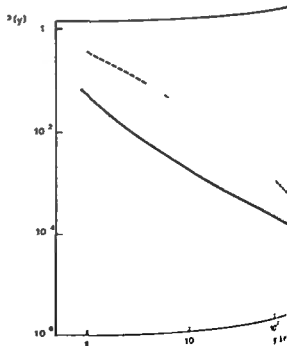


Fig. 5. Probability distribution of lineal energy per unit path length based on secondary electrons emission from a 95.5 nm thick carbon foil at 6 keV. (—) $P(0)=0.99$ (---) $P(0)=0.99$.

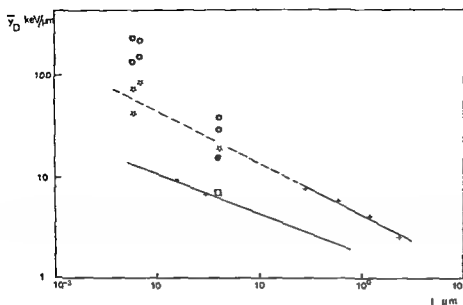


Fig. 6 Dose mean of lineal energy versus mean chord length in unit density material. ^{60}Co γ rays (\circ) BENGTSSON & LINDBORG (1974) ^3H β -rays ($+$) BRABY & ELLETT (1971) 7 keV electrons

(\bullet) and ^{87}Sr - ^{90}Y β rays (\square) calculated from LLACER & GARWIN (1969b) Calculations (\circ) reflection) Calculations (\circ) transmission) C corresponds to $l=6$ nm Al to $l=7$ nm LiF to $l=4$ nm

the entrance surface of an absorber and illustrates how the distribution can vary with the nature of the absorbing material. It will be noted that the linear dimension of the sensitive volume varies with the material because of differences in thickness of the escape zone. Fig. 5 presents data relevant to secondary electrons emitted from the exit surface of an absorber and shows how the distribution can vary with the energy of the incident electron. The incident energy of the primary electrons is indicated on Fig. 5 but the energy of the electrons passing through the escape zone on the exit side of the foil will of course be less than this.

These data form the basis for calculating the dose mean and the frequency mean of the lineal energy and the relative variance. These values are presented in Table 3. Where data in Table 3 (and also Figs. 5-7) are given for transmission, the calculations have been undertaken for the foil thickness employed by KANTER and LLACER & GARWIN (1969b). They were 95.5 nm, 50 nm and 64.8 nm for carbon, lithium fluoride and aluminium, respectively. The quantities in Table 3 are defined as follows:

$$y_F = \frac{W_{ef}}{l} \frac{\sum_0 i n_i}{\sum_0 n_i} = \frac{W_F}{l} \left\{ \frac{0}{\sum_0 n_i} + \frac{\sum_1 i n_i}{\sum_1 n_i} \right\} = \delta \frac{W_F}{l} \quad (6)$$

$$y_{F1} = \frac{W_F}{l} \frac{\sum_1 i n_i}{\sum_1 n_i} \quad (7)$$

$$y_D = \frac{W_{ef}}{l} \frac{\sum_0 i n_i}{\sum_0 n_i} \quad (8)$$

$$y_{F1} = \frac{y_D}{y_F} \sim 1 \quad (9)$$

Where n_i events result in the emission from the surface of i secondary electrons, δ is the average yield of secondary electrons per primary electron and l is the mean chord length across the escape zone.

The first term in the summation for y_F represents an event which does not result in the emission of a secondary electron (this may occur either because the primary electron did not generate any secondary electrons within the escape zone or because secondary electrons which were generated failed to cross the surface potential barrier) and the second term the number of events which do.

A large difference exists between the values of y_F and y_{F1} . This is because there are many events which do not lead to the emission of a secondary electron and these events heavily weight the spectrum. For carbon and aluminium the probability per primary particle that a secondary electron will not be emitted is 0.95 and for lithium fluoride 0.80 for 6 keV primary electrons. The mean lineal energy is always larger in the case of the transmission data for three reasons. First, some input data for the calculation were based on curve fitting of transmission

Table 3

Mean values and relative variance of the single event distribution

Material	Primary electron energy E_0 (keV)	Mean lineal energy (keV/ μ m)			Relative variance V_1
		y_F	$y_{1.1}$	y_D	
Reflection					
Carbon	6	1.0	23.5	71	70
Lithium fluoride	6	2.1	9.7	19	8
Aluminium	6	1.2	17.9	87	67
Transmission					
Carbon	6	2.3	69.0	225	96
	60	0.2	55.0	138	690
Lithium fluoride	6	2.4	17.5	38	11
	60	0.3	12.3	78	176
Aluminium	6	2.6	57.2	214	81
	60	0.2	50.5	143	714

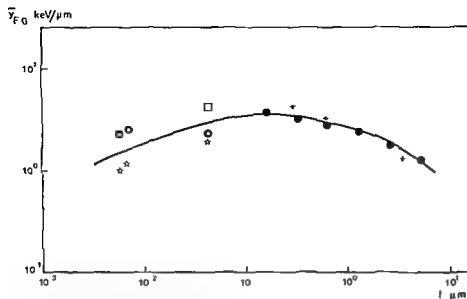
measurements and relative to reflection measurements (HOLZL & JACOBI 1969) the calculation tends to give low values for the yield from the incident surface. Secondly the primary electrons will have a lower energy on reaching the exit surface of the foil. Thirdly the primary electrons are normally incident on the escape zone of the entry surface but will tend to be more isotropic as they cross the escape zone of the exit surface. In contrast to carbon and aluminium the value of the mean lineal energy for lithium fluoride obtained from reflection and transmission calculation is not very different. This is because the foil thickness for lithium fluoride is 50 nm and the escape zone is 24 nm. Thus the entry and exit escape zone are adjacent or even overlapping for lithium fluoride whereas they are widely separated for carbon and aluminium. It will be noted that for these small volumes the relative variance is extremely large.

The dose mean of lineal energy y_D obtained from conventional microdosimetric measurements is compared with values obtained from secondary electron emission in Fig. 6. For low energy electrons the experimental results obtained by proportional counter techniques (BRABY & FLEET 1971) using ^{57}Fe β rays are compared with the data in Table 3 and the secondary electron emission measurement by LIACER & GARWIN using 7 keV electrons. The measurement in lithium fluoride is very close to the value calculated by the theory of McDONALD et

coll for reflected secondary electrons. The position of the secondary electron points is of course determined by the thickness of the escape zone for particular material. Thus the lowest value of mean chord length (i.e. smallest volume) is for carbon 6 nm and aluminium 7 nm. If the proportional counter results are extrapolated linearly they fit in the graphs through the results obtained by secondary electron emission giving good agreement with the measurement by LIACER & GARWIN and the theory of McDONALD et coll for reflected secondary electrons.

The experimental results obtained by BENGTSSON & LINDBORG (1974) with the electrons produced by 1.25 MeV ^{60}Co γ rays are compared with a value derived from the secondary electron emission measurement by LIACER & GARWIN using ^{57}Fe β rays. The measurements by BENGTSSON & LINDBORG extend down to 7 nm mean chord length and therefore no extrapolation is necessary to bridge the measurement on lithium fluoride by LIACER & GARWIN. The agreement is good although the approximations in the estimation of mean chord length have to be borne in mind. Agreement for both high and low energy electrons is particularly interesting, when it is recalled that secondary electron emission simulates a very thin layer of material while conventional microdosimetric techniques simulate spheres or cylinders.

Further data are required on y_D values for



Frequency mean of lineal energy versus mean chord length density material H β rays (+) BRABY & ELLETT (1972) rays (●) KLIAGUA & DYORAK (1978) 7 keV electrons (□)

calculated from LLACER & GARWIN (1969b) Calculations for C Al and LiF (□ ●) as in Fig 6

ly electrons or roentgen and it is possible that a technique used by BENGTSOON & LINDBORG could be applied to secondary electron emission devices. An experimental approach to this has been undertaken (FORSBERG 1978) and further experiment is being made.

The frequency mean of lineal energy y_F obtained from conventional microdosimetry measurements is compared with values obtained from secondary electron emission in Fig 7 for low energy protons. The conventional measurements by BRABY & ELLETT with $^3\text{H}\beta$ rays and by KLIAGUA & DYORAK (1978) with electrons produced by 11 keV x-ray tubes show good agreement; the frequency mean of the lineal energy increases slowly with increasing volume. The secondary electron emission data for 6 keV electrons from Table 3 calculated by McDONALD's theory and the measurement for 7 keV electrons by LLACER & GARWIN are also shown. A continuous line has been plotted through the data. At mean chord lengths below 100 nm the frequency mean of the lineal energy decreases slowly with decreasing volume. This reflects the influence of the zero events that is particles passing through the volume without resulting in any escape of secondary electrons. It has been argued in the foregoing that secondary electron emission techniques can provide useful microdosimetric data. The results are not very sensitive to the choice of ϵ and P (ϵ_0 , 0) but will much uncertainty arise from the choice of l

and Z . The greatest uncertainty in these calculations will arise from the choice of l , which will influence the shape of some distributions and W_{SE} , which will not influence the shape of the distributions but will affect the absolute values and hence the mean value quoted. The maximum uncertainty in the absolute values is estimated at about 50 per cent. CHMELEVSKY & KELLERER (1977) have recently undertaken computation of microdosimetric functions for sites of 5, 10 and 100 nm diameter for protons of energies 0.5, 5 and 20 MeV. While not directly comparable with the present calculations, some confirmation of the trend of the y_0 values in Fig 6 can be found. For protons with a comparable stopping power as 6 keV electrons they also got an increasing y_0 at decreasing object sizes. However, the burden of this report was not to produce absolute values but to illustrate some of the potentials of this alternative approach to microdosimetry.

In conclusion the authors wish to emphasize three aspects of this technique which may offer some advantages over other physical models of biologic systems. First, the linear dimensions (10 nm) are comparable with what is believed to be the dimensions of biologic targets. Secondly, the shape is an extended layer which would simulate the situation where the sensitive structures are distributed in these layers, for example in the vicinity of nuclear membranes (ALPER 1969). Thirdly, another interesting biologic application should be the use of

secondary electron emission to examine the two target theory. It has been proposed by several authors that certain biologic effects may result from energy depositions taking place in two different targets sublesions (NEARY 1965, ROSSI 1968, ALPER). NEARY suggested that these targets should be in the nanometer region and a separation distance of $0.2 \mu\text{m}$. KELLERER & ROSSI (1971) concluded that cells of higher organisms are injured by radiation through production of an ionisation in each of two targets of nanometer size with an interaction distance of micrometers. ALPER proposed that cell death may occur if energy depositions are taking place both in the DNA and the nuclear membrane. Most recently KELLERER & ROSSI (1978) have presented a generalised formulation of dual radiation action. Aspects of the situations could be simulated by registering the secondary electrons liberated by the same primary particles from escape zones (having dimensions of about 10 nm) on the entry and exit sides of a foil having dimensions of $0.2 \mu\text{m}$ or above.

SUMMARY

The potential of secondary electron emission as a basis for microdosimetric analysis is explored. Secondary electron emission phenomena are compared with gaseous ionisation and a brief review of work on the stochastic nature of secondary emission is presented. Input data are selected and calculations undertaken to obtain lineal energy spectra. The frequency and dose mean of lineal energy obtained from these calculations in various volumes are compared with results obtained with proportional counters. Some biologic applications of this approach to microdosimetry are discussed.

ACKNOWLEDGEMENTS

The authors wish to acknowledge several helpful discussions with Dr I. R. McDonald, Dr L. Lindborg, and Dr J. A. Simmons.

REFERENCES

- ALPER, T. Mechanisms of lethal radiation damage to cells. In: *Proceedings of the second Symposium on Microdosimetry*, Verbania, p. 5. Eur-4452 Commission of the European Communities, Luxembourg, 1969.
- BIRCHSON, I. G. and LINDMOR, U. I. R. Comparison of pulse height analysis and variance measurements for the determination of dose mean of specific energy. In: *Proceedings of the fourth Symposium on Microdosimetry*, Verbania, p. 832. Eur-5122 Commission of the European Communities, Luxembourg, 1974.
- BIRKHOF, R. D., TURNER, J. I., ANDERSON, V. I., FLOLA, I. M. and HAMM, P. N. The determination of IIT spectra from energy proportional pulse height measurements. I. Track length distributions in ca. Hlth Phys. 18 (1970): 1.
- BICHSEL, H. Charged particle interactions. Ch. 7. Radiation dosimetry. I, p. 157. Edited by F. H. and W. C. Roessch. Academic Press, New York & San Francisco, London, 1968.
- BRABY, L. A. and FLETT, W. H. Ionization in volumes irradiated by energetic photons. Org. & University Report A0731709 (1971).
- BROSHSTEIN, I. M. and FRAMIN, B. S. Inelastic scattering of electron emission from certain metals and semiconductors. Sov. Phys. Solid State 3 (1961): 1178.
- and SECAR, R. B. Inelastic scattering of electrons: secondary electron emission in certain metals. Sov. Phys. Solid State 1 (1968): 1356.
- BURLIN, T. F. The characteristics of secondary electron emission and some potential applications to microdosimetry. In: *Proceedings of the fourth Symposium on Microdosimetry*, Verbania, p. 35. Eur-5122 Commission of European Communities, Luxembourg, 1974.
- CAFOTTA, A. A., CARTER, I. N., DELANEY, C. F. G. & McDONALD, I. R. The attenuation and backscattering of electron beams by thin films. J. Phys. D: Appl. Phys. 4 (1975): 1210.
- CHMETEVSKY, D. and KELLERER, A. M. Computer simulation of microdosimetric distributions for small sites. Radiat. Environ. Biophys. 14 (1977): 173.
- DELANEY, C. F. G. and WATSON, P. W. Measurement of the statistics of secondary electron emission. III. Trans. nucl. Sci. Nucl. 13 (1966): 747.
- DIETZ, L. A., HANRAHAN, I. R. and HANCOCK, A. B. Secondary electron response of a porous KCl transducer: a dynode and application of Polya Statistics to pulse counting in an electron multiplier. Rev. Sci. Instrum. 37 (1966): 176.
- DIATOWITSKAJA, B. I. Secondary emission of atomic caesium cathodes. Dokl. Akad. Nauk SSSR (1948): 641.
- DVORAK, R. F. Event distributions for monochromatic photons. Annual Report of Research Project R-4, Res. Lab. Colombia Univ. 0003741-4 (1975): 41.
- FITTING, H. I., GRAEFEN, H., WILD, W. and NEUBAUER, M. Multiple scattering of fast electrons and secondary electron generation within semi-infinite targets. Phys. D: Appl. Phys. 9 (1976): 2499.
- FORSBERG, B. An experimental approach to determining microdosimetric quantity for a nanometer thick target. SSI report 1978-034. National Institute of Radiation Protection, Stockholm, 1978.
- GREEN, M. A. Monte Carlo calculation of the probability of characteristic X-ray production in a target. Proc. physiol. Soc. 82 (1973): 64.
- GRUBER, H. Untersuchungen der Wahrscheinlichkeitsverteilungen der Anzahl Atomteilchen, die Elektronen aus bedeckten Festkörperschichten ausstrahlen. J. Mass. Spectrum. Ion Phys. 10 (1977): 43.
- HOLST, I. and JACOB, K. Sekundärelektronen aus dünnen Kohlenstofffolien in Transmissions- und Reflexion. Surf. Sci. 14 (1974): 349.
- International Commission on Radiation Units and

- ents (ICRU) Linear energy transfer Report 16
ington D C 1970
- II Energy dissipation and secondary electron
ion in solids Phys Rev 121 (1961) 677
- III R A M and ROSSI H II RBE and the primary
anism of radiation action Radiat Res 47 (1971)
- generalized formulation of dual radiation action
t Res 75 (1978) 471
- IV P and DVORAK M Microdosimetric measure-
s of ionization by monoenergetic photons Radiat
73 (1978) 1
- I and GARWIN E L (a) Electron secondary
ion from alkali halides J appl Phys 40 (1969)
- (b) Statistics of transmission secondary emission
thin films of alkali halides J appl Phys 40
9 3936
- V FLL W I HUBBELL H H and BIRKHOFF R D
ing down spectrum of 19 Au beta rays in gold Hlth
1⁷ (1966) 693
- VI D I R LANKA M and DELANFY C F G
attenuation and backscattering of electron beams
in films J Phys D Appl Phys 4 (1971) 1210
- Electron emission from alkali halides under soft
X ray bombardment J Phys D Appl Phys 6 (1973)
87
- NAKHODKIN N G and ROMANOWSKY V A Variation of
the secondary emission coefficient of KCl with the
layer thickness Izv Akad Nauk SSR 22 (1958) 457
- NEARY G I Chromosome aberrations and the theory of
RBE 1 General considerations Int J radiat Biol 9
(1965) 477
- OSTROUKHOV A A Inelastic scattering of medium ener-
gy electrons in the continuous energy loss approxima-
tion Sov Phys Solid State 9 (1967) 1369
- PRESCOTT J R A statistical model for photomultiplier
single-electron statistics Nucl Instrum Meth 39
(1966) 173
- REIMER L and DRESCHER H Secondary electron emis-
sion of 10-100 keV electrons from transparent films of
Al and Au J Phys D Appl Phys 10 (1977) 805
- ROSSI H H Specification of radiation quality Radiat
Res 10 (1959) 522
- Role of associated absorption events in dendicular
radiation injury In Biophysical aspects on radiation
quality p 161 IAEA Vienna 1968
- WILCOCK W L and MILLER D E Statistics of transmit-
ted secondary electron emission Adv El and El
Phys 28A (1969) 513

SIGNIFICANCE OF QUANTUM FLUCTUATIONS
IN ROENTGEN IMAGING

KARL GUSTAV STRID

Optimisation of roentgen diagnostic techniques implies the development of procedures by means of which the desired diagnostic image quality is produced with the further requirement that the radiation dose absorbed by the patient be kept at a minimum. Image quality is limited by a number of factors which may be grouped under a few headings: viz (a) geometric unsharpness (blurring due to small spot size, motion blurring, light spread in detecting layers, etc.), (b) dynamic limitations (visibility thresholds), and (c) statistical limitations (quantum noise, lack of elementary receptors in detecting layers). Unsharpness is readily describable by means of the optical transfer function of the imaging system (MORGAN 1962, SCHÖBER & HÖRT 1963), whereas dynamic limitations are inferred from the overall system sensitometric function, inclusive of the contrast threshold of the human eye inspecting the final image (BLACKWELL 1946). The significance of photon statistics was pointed out by STURM & MORGAN (1949), the fluctuations giving rise to quantum mottle (CLEARE et coll. 1962, ROSS et coll. 1963). Lack of receptors (e.g. silver halide grains) in the detecting layer further contributes to the resulting image mottle (SELIN & REICHMANN 1977), which implies that secondary radiation must be kept at a minimum (SELIN et coll. 1975, STRID 1976, REICHMANN & STRID 1979), contrary to the view held by REISS (1963). The relation between image quality and patient dose was examined experi-

mentally by REICHMANN & ÅSTRAND (1979) and theoretically analysed by MOTZ & DAVOS (1978).

Within the scope of a program for optimising roentgen imaging techniques, a series of mathematical investigations has been initiated. The work will concern the statistics of the radiation field, the interaction between roentgen radiation and photographic type detectors, and the interplay of unsharpness and quantum fluctuations.

As a first part of the work, the present report furnishes an analysis of quantum fluctuations in the radiation field, proper to some implications with regard to image recording, also being discussed; however, the behaviour of photographic type detectors will not be treated. In addition, the mathematical framework to be employed in forthcoming reports is provided. Therefore, elements of the theory of stochastic variables, somewhat beyond the scope of the present report, have been compiled in an Appendix.

List of symbols

Other symbols than those listed below may occasionally be used in the text. Additional symbols are used in the Appendix.

A Area of object detail to be detected as projected on the recording plane, unit 1 m

C	Radiation contrast
d	Linear extent of object detail to be detected unit $l\ m$
D	Radiation dose unit $l\ Gy$
k	Constant of proportionality
Λ	Number of photons
p	Probability of a photon process
$P(x)$	Probability that the event x will take place
S	Signal to-noise ratio
T	Transmittance of secondary screening device
$var(x)$	Variance of the quantity x
η	Detective quantum efficiency
ν	Repeatability (spatial frequency) of object structure to be resolved unit $l\ m^{-1}$
$\sigma(x)$	Standard deviation of the quantity x
Σ	Selectivity of secondary screening device
Φ	Photon fluence unit $l\ m$
ψ	Relative fluence of secondary radiation

Subscripts

D	Detector (recording device)
p	Primary radiation
s	Secondary radiation
t	Total radiation field
0	Incident radiation

Primed quantities e.g. S_1 refer to the radiation field behind the secondary screening device whereas double primed ones e.g. S_2 refer to the response of the detector. Angle brackets e.g. $\langle x \rangle$ denote the mean value of the quantity described by the enclosed symbol.

Basic considerations

A radiosopic situation is considered where an object is exposed to roentgen radiation of photon fluence Φ_0 (Fig. 1). For the sake of simplicity—in order to arrive at a mathematical model of instructive clarity—the radiation is assumed to be monoenergetic i.e. composed of photons of equal energy content. This restriction may be easily relieved by replacing fluence with its differential spectral distribution and integrating the relevant quantities over the entire spectrum; however the clarity of the model would then be lost.

On passage through the object the radiation is attenuated and behind the object a radiation field appears viz. the primary radiation relief in which the photon fluence Φ_p is modulated with information regarding the distribution of matter in the ob-

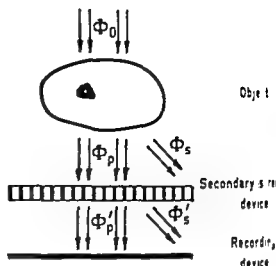


Fig. 1. Flow of roentgen energy in radiology. Incident field of photon fluence Φ_0 is modulated by the object into an informative field of fluence Φ_p and a non-informative field of fluence Φ_s . Secondary screening modifies the Φ_s values to Φ_s' and Φ_s'' respectively.

ject. Moreover due to scattering and fluorescence secondary radiation arises in the object as another field of photon fluence Φ_s . For the present analysis this field is assumed to be homogeneous over the imaging area considered. The resulting secondary radiation relief will impair the paired imaging as compared to the primary radiation alone.

In order to restore image quality some kind of discriminating device is inserted into the secondary radiation relief to produce a modified radiation field with fluence values Φ_p and Φ_s' respectively, this field being intercepted by the recording device so as to produce the image.

Formation of contrast

If the radiosopic system is intended to resolve object details of a characteristic extent d (Fig. 2) it is relevant to consider the imaging of a periodic object of repeatability (spatial frequency) ν . In more specific terms to analyse the contrast between two squares 1 and 2 of the same area A (Fig. 2) one in a region of high transmittance and the other in a region of low transmittance of the object.

The transmitted primary fluence is assumed to be Φ_{p1} and Φ_{p2} respectively. Due to the nature of the radiation these quantities refer to the average flow of photons (cf. ICRU 1971). The

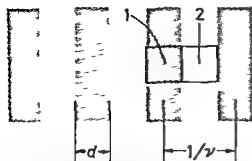


Fig. 1. Schematic representation of a periodic object of regularity ν . For the structure to be resolved by radioscopy the imaging system must yield significantly different responses for areas 1 and 2 of area $A = d^2 = 1/\nu^2$ located respectively in regions of low and high transmittance

the object to modulate the radiation field is then statistically described by the primary radiation contrast

$$C_1 = \left| \frac{\Phi_1 - \Phi_0}{\Phi_0} \right| \quad (1)$$

here

$$\Phi_0 = \frac{1}{2}(\Phi_{p1} + \Phi_p) \quad (2)$$

the secondary radiation presents the fluence Φ which is proportional to the primary fluence

$$\Phi_s = \psi \Phi_p \quad (3)$$

hereby the relative fluence of secondary radiation is defined. The resulting radiation contrast is then changed from C_p to

$$C_1 = \left| \frac{\Phi_1 - \Phi_0}{\Phi_0 + \Phi_s} \right| = \frac{C_1}{1 + \psi} \quad (4)$$

This contrast reduction however does not exhaustively account for the resulting image impairment since the statistical properties of roentgen radiation have been entirely neglected. Thus the conventional treatments e.g. by OOSTERKAMP (1946) HONDIUS BOLDINGH (1960 1964) and REISS give an incomplete description of the effects of secondary radiation.

Randomness of roentgen radiation

The emission of photons in the radiation source takes place in a completely random way i.e. the moment and the direction of emission of a particular photon are completely unrelated to those of any

photon emitted previously. Roentgen photon emission is a stochastic process of the Poisson type (cf Appendix). Absorption, scattering and secondary emission by the radioscopic object are likewise random processes. Indeed the conversion in the object of the incident radiation field to one field of informative photons (primary radiation) and another of non-informative ones (secondary radiation) are binomially distributed stochastic processes (cf Appendix) making the resulting fluxes Poisson distributed as well. No exact a priori predictions may be made as to the numbers of photons of primary and secondary origin propagating through a given region of space during a particular interval of time. However the average values of these numbers can be calculated provided the proper physical and geometric characteristics of the radiation source and the radioscopic object are known.

Signal to-noise ratio

Consider a region in the object forming a cylinder along the direction of incident radiation of projected area A . If the probability of undisturbed transmission of a photon through the object is p_p , the fluence of primary photons leaving the object is $\Phi_p = p_p \Phi_0$, Φ_0 being the fluence of incident photons and the mean number of photons transmitted through the region of area A is

$$\langle N_p \rangle = \Phi_p A = p_p \Phi_0 A \quad (5)$$

with the standard deviation

$$\sigma(N_p) = \Phi_p A \quad (6)$$

which follows from eq. (A.19) (Appendix). Similarly for a laterally extended and reasonably homogeneous object the mean number of secondary photons emitted towards the corresponding area in the recording plane is

$$\langle N_s \rangle = \Phi_s A = \psi \Phi_0 A \quad (7)$$

with the standard deviation

$$\sigma(N_s) = \sqrt{\Phi_s} A \quad (8)$$

assuming p_s to be the probability of an incident photon being turned into a secondary one travelling towards the recording plane.

Now consider the periodic object in Fig. 2. The primary radiation field is modulated by the object the primary contrast being defined by eq. (1). Since small variations in object transmittance are of the utmost importance in diagnostic radiology and moreover present the greatest recording difficulties the assumption of $C_p \ll 1$ will be used throughout the present analysis.

Primary signal to noise ratio The recording plane areas corresponding to object regions 1 and 2 are passed by N_{p1} and N_p photons respectively. The information relating to the difference in object transmittance is given by the difference

$$\delta N_p = N_{p1} - N_p \quad (9)$$

which is thus the signal to be recorded. In an actual recording experiment the value of δN_p is not a priori predictable, δN_p being a stochastic variable. Thus the experiment will not generally yield the mean value $\langle \delta N_p \rangle$ but some more or less deviating value. The more the value actually recorded is expected to deviate from $\langle \delta N_p \rangle$ the less significance will have to be ascribed to the recorded value. A measure of the expected deviation, the noise of the recording, is the standard deviation $\sigma(\delta N_p)$. The significance of the recording is suitably described by a signal to noise ratio defined for the primary radiation field as

$$S_p = \frac{|\langle \delta N_p \rangle|}{\sigma(\delta N_p)} \quad (10)$$

From eqs. (A 11), (9), (5) and (1) it follows that

$$\begin{aligned} \langle \delta N_p \rangle &= \langle N_{p1} \rangle - \langle N_p \rangle \\ &= (\Phi_{p1} - \Phi_p)A = C_p \Phi_p A \end{aligned} \quad (11)$$

whereas (A3), (A13), (6) and (2) yield

$$\begin{aligned} \sigma(\delta N_p) &= \sqrt{\sigma(N_{p1}) + \sigma(N_p)} \\ &= \sqrt{(\Phi_{p1} + \Phi_p)A} = \sqrt{2\Phi_p A} \end{aligned} \quad (12)$$

Then the primary signal to noise ratio obtains as

$$S_p = C_p / \sqrt{2\Phi_p A} \quad (13)$$

which expresses the fundamental intrinsic noisiness of the primary radiation itself. No actual recording process can yield a higher value for the signal to noise ratio than S_p of eq. (13). Most re-

cording processes of a photographic type start from this ideal limiting case.

Detectability limit It was suggested by L. I. (1948) (cf. STURM & MORGAN, MORGAN, MOTZ & DANOS) that the threshold value of signal to noise ratio for visual detection was ≈ 5 . Suppose that an object region of projected area $A = 1 \text{ mm}^2$ yielding a primary contrast $C_p = 0.1$ is to be detected. Then for the limiting case of detection eq. (13) yields the necessary value for the photon fluence $(\Phi_p)_{\min} \approx 2 \times 10^5 / (1 \text{ mm}^2 \times 0.1) = 2 \times 10^5 \text{ mm}^{-2}$.

Still there exists a finite probability for the contrast to remain undetected or be detected erroneously so that a dark object detail appears bright in the image. Suppose that $\Phi_{p1} > \Phi_p$. In an actual recording case due to quantum fluctuations $N_{p1} \leq N_p$ may be observed. This implies $\delta N_p \leq 0$ or $|\delta N_p - \langle \delta N_p \rangle| \geq \langle \delta N_p \rangle$. From Chebyshev's inequality (A 4) it follows that

$$P(|\delta N_p - \langle \delta N_p \rangle| \geq \langle \delta N_p \rangle) < \left(\frac{\sigma(\delta N_p)}{\langle \delta N_p \rangle} \right)^2 = 1/5$$

where the definition (10) has been applied. However, about one half of these cases correspond to instances of enhanced contrast and thus $P \approx 1/2 S_p$.

Obviously, in the present example the probability of undetected or reversed contrast is as great as $1/50$.

Signal to noise ratio in presence of secondary radiation Consider now the case where the recording plane intercepts secondary radiation. Assume the object to be laterally extended and sufficiently homogeneous for the secondary radiation to be uniform in the area surrounding the recording plane region where the primary radiation from regions 1 and 2 is being detected. The corresponding recording plane areas are now passed by N_{s1} and $N_s = N_{s1} + N_{s2}$ photons respectively, the secondary radiation being spatially uniform.

$$\langle N_{s1} \rangle = \langle N_s \rangle = \psi \Phi_p A$$

where ψ is defined by eq. (3). The signal $\delta N_s = N_{s1} - N_s$. Application of eqs. (A 11) and (12) gives

$$\begin{aligned} \langle \delta N_s \rangle &= \langle N_{s1} \rangle - \langle N_s \rangle = \langle N_{s1} \rangle - \langle N_{s1} + N_{s2} \rangle \\ &= \langle N_{s1} \rangle - \langle N_{s1} \rangle - \langle N_{s2} \rangle = -\langle N_{s2} \rangle \\ &= -C_p \Phi_p A \end{aligned}$$

the same expression as eq (11) for primary radiation alone. For the variance of δN eqs (A 13) (8) and (14) yield

$$\text{var}(\delta N) = \text{var}(N_{p1}) + \text{var}(N_{s1}) + \text{var}(N_p) + \text{var}(N_s) \\ = \langle N_{p1} \rangle + \langle N_p \rangle + 2\langle N \rangle$$

by the application of eqs (2) and (3)

$$\sigma(\delta N) = \sqrt{2(\Phi_p + \Phi)A} = \sqrt{2\Phi_p A(1+\psi)} \quad (16)$$

comparison with the case of no secondary radiation described by eq (12) noise has been increased a factor $\sqrt{1+\psi}$

The signal to noise ratio as defined by (10) is extended to the case of secondary radiation in (1) (15) and (16) it is found that

$$S_1 = C_1 \sqrt{\frac{1}{2} \Phi_p A / (1+\psi)} \quad (17)$$

the signal to noise ratio has deteriorated by a factor $1/\sqrt{1+\psi}$ due to the presence of secondary radiation. Contrary to commonly held views (e.g. ELISS) the information thus lost cannot be recovered by any post recording contrast-enhancing procedure: the loss is irreversible.

Effects of secondary screening

A secondary screening device is a scattered ray grid inserted into the modulated radiation field behind the object in order to reduce the relative fluence of secondary radiation (cf. STRID). The reduction from the value ψ to the value ψ_s is described by the device's selectivity

$$\Sigma = \psi / \psi_s \quad (18)$$

however part of the informative (primary) fluence will be lost in the device: changing this fluence from Φ_p to

$$\Phi_p = T_p \Phi_p \quad (19)$$

which defines the device's transmittance for primary radiation T_p .

Since the screening device functions by means of stochastically distributed stochastic processes (cf. Appendix) the fluences after secondary screening are still Poisson-distributed. Therefore eqs (15) and (16) still hold true provided that ψ is replaced by

Φ_p and ψ by ψ_s . The signal to noise ratio is then obtained as

$$S_1 = C_1 \sqrt{\frac{1}{2} T_p \Phi_p A / (1+\psi_s)} \quad (20)$$

Suppose that in a severe case e.g. abdominal radiography $\psi \approx 7$ (WILSEY 1921). Then with no secondary screening $S_1 \approx S_p / \sqrt{8} = 0.35 S_p$. An efficient optimised grid will perform $\Sigma \approx 15$ with $T_p \approx 0.50$ (STRID). Insertion of such a grid will improve the signal to noise ratio to $S_1 \approx 0.58 S_p$ as is seen from eq (20). The improvement may appear marginal. However when the characteristics of the recording devices commonly used are taken into account the efficient grid will come out still more favourable.

Aspects of recording devices

Dynamically limited detectors The expressions (17) and (20) for the signal to-noise ratio contain the primary fluence Φ_p . Only if the recording device is free from dynamic limitations can the signal to noise ratio values be compared for the same value of Φ_p . Contrary to these detectors commonly used e.g. film screen combinations or television cameras are dynamically limited. Thus a certain threshold fluence is required for a response to be produced and fluence in excess of a certain saturation value will not produce any further response. The radiation relief with all its modulation detail must thus be contained within the dynamic limits of the recording device.

Consider a detector with the simple characteristic of Fig 3. This is a first-order model of a photographic recording device. In order that a useful image may be produced the total fluence into the device $\Phi_t = \Phi_p + \Phi_s = \Phi_p(1+\psi)$ must fall within the responsive range of the device. Therefore signal to noise ratio values must be compared at some optimum value Φ_0 in the central part of the responsive range. The overall signal to noise deterioration caused by secondary radiation is thus demonstrated by the comparison of S_p relating to $\Phi_p = \Phi_0$ with S_1 relating to $\Phi_p(1+\psi) = \Phi_0$. Then

$$S_p = C_1 \sqrt{\frac{1}{2} \Phi_0 A}$$

$$S_1 = C_1 \sqrt{\frac{1}{2} \Phi_0 A / (1+\psi) / \sqrt{1+\psi}}$$

and consequently

$$S_1 / S_p = \frac{1}{1+\psi} \quad (21)$$

Now consider the periodic object in Fig 2. The primary radiation field is modulated by the object the primary contrast being defined by eq (1). Since small variations in object transmittance are of the utmost importance in diagnostic radiology and moreover present the greatest recording difficulties the assumption of $C_p \ll 1$ will be used throughout the present analysis.

Primary signal to noise ratio. The recording plane areas corresponding to object regions 1 and 2 are passed by N_{p1} and N_{p2} photons respectively. The information relating to the difference in object transmittance is given by the difference

$$\delta N_p = N_{p1} - N_{p2} \quad (9)$$

which is thus the signal to be recorded. In an actual recording experiment the value of δN_p is not a priori predictable δN_p being a stochastic variable. Thus the experiment will not generally yield the mean value $\langle \delta N_p \rangle$ but some more or less deviating value. The more the value actually recorded is expected to deviate from $\langle \delta N_p \rangle$ the less significance will have to be ascribed to the recorded value. A measure of the expected deviation the noise of the recording is the standard deviation $\sigma(\delta N_p)$. The significance of the recording is suitably described by a signal to noise ratio defined for the primary radiation field as

$$S_p = \frac{|\langle \delta N_p \rangle|}{\sigma(\delta N_p)} \quad (10)$$

From eqs (A 11) (9) (5) and (1) it follows that

$$\begin{aligned} \langle \delta N_p \rangle &= \langle N_{p1} \rangle - \langle N_{p2} \rangle \\ &= (\Phi_{p1} - \Phi_{p2})A = C_p \Phi_p A \end{aligned} \quad (11)$$

whereas (A3) (A13) (6) and (2) yield

$$\begin{aligned} \sigma(\delta N_p) &= \sqrt{\sigma^2(N_{p1}) + \sigma^2(N_{p2})} \\ &= \sqrt{(\Phi_{p1} + \Phi_{p2})A} = \sqrt{2\Phi_p A} \end{aligned} \quad (12)$$

Then the primary signal to noise ratio obtains as

$$S_p = C_p \sqrt{1/\Phi_p A} \quad (13)$$

which expresses the fundamental intrinsic noisiness of the primary radiation itself. No actual recording process can yield a higher value for the signal to noise ratio than S_p of eq (13). Most re-

cording processes of a photographic type start from this ideal limiting case.

Detectability limit. It was suggested by J. MOTZ & DANOS (1948) (cf STURM & MORGAN MORGAN MOTZ & DANOS) that the threshold value of signal to noise ratio for visual detection was ≈ 5 . Suppose that an object region of projected area $A = 1 \text{ mm}^2$ yielding a primary contrast C_p is to be detected. Then for the limiting case of detection eq (13) yields the necessary value of the photon fluence $(\Phi_p)_{\min} \approx 2 \times 5 / (1 \text{ mm}^2 \times 0.5 \times 10^{-2} \text{ mm}^2)$.

Still there exists a finite probability for the contrast to remain undetected or be detected erroneously so that a dark object detail appears bright in the image. Suppose that $\Phi_{p1} > \Phi_{p2}$ in an actual recording case due to quantum fluctuations $N_{p1} \leq N_{p2}$ may be observed. This implies that $\delta N_p \leq 0$ or $|\delta N_p - \langle \delta N_p \rangle| \geq \langle \delta N_p \rangle$. From Chebyshev's inequality (A 4) it follows that

$$P(|\delta N_p - \langle \delta N_p \rangle| \geq \langle \delta N_p \rangle) < \left(\frac{\sigma(\delta N_p)}{\langle \delta N_p \rangle} \right)^2 = 1/S_p^2$$

where the definition (10) has been applied. However, about one half of these cases correspond to instances of enhanced contrast and thus $P(\delta N_p < 0) \approx 1/2S_p$.

Obviously, in the present case the probability of undetected or reversed contrast is as great as $1/50$.

Signal to noise ratio in presence of secondary radiation. Consider now the case where the recording plane intercepts secondary radiation as well. Assume the object to be laterally extended and sufficiently homogeneous for the secondary radiation to be uniform in the area surrounding the recording plane region where the primary radiation is recorded. Regions 1 and 2 are being detected. The corresponding recording plane areas are now passed by N_{p1} and $N_{p2} \approx N_{p1} + N_{s2}$ photons respectively, the secondary radiation being spatially uniform.

$$\langle N_{p1} \rangle = \langle N_{p2} \rangle = \psi \Phi_p A$$

where ψ is defined by eq (3). The signal to noise ratio S_p is defined by eq (1). Application of eqs (A 11) (12) gives

$$\begin{aligned} \langle \delta N_p \rangle &= \langle N_{p1} \rangle + \langle N_{s1} \rangle - \langle N_{p2} \rangle - \langle N_{s2} \rangle \\ &= \langle N_{p1} \rangle - \langle N_{p2} \rangle = (\Phi_{p1} - \Phi_{p2})A \\ &= C_p \Phi_p A \end{aligned}$$

the same expression as eq (11) for primary radiation alone. For the variance of δN eqs (A 13) (8) and (14) yield

$$\sigma(\delta N) = \text{var}(N_{p1}) + \text{var}(N_{s1}) + \text{var}(N_p) + \text{var}(N_s) \\ = \langle N_{p1} \rangle + \langle N_{s1} \rangle + 2\langle N \rangle$$

by the application of eqs (2) and (3)

$$\sigma(\delta N) = \sqrt{2(\Phi_p + \Phi_s)A} = \sqrt{2\Phi_p A(1 + \psi)} \quad (16)$$

comparison with the case of no secondary radiation described by eq (12) noise has been increased a factor $\sqrt{1 + \psi}$

The signal to noise ratio as defined by (10) is extended to the case of secondary radiation in (1) (15) and (16) it is found that

$$S_1 = C_p \sqrt{\frac{1}{2} \Phi_p A / (1 + \psi)} \quad (17)$$

the signal to noise ratio has deteriorated by a factor $1/\sqrt{1 + \psi}$ due to the presence of secondary radiation. Contrary to commonly held views (e.g. [15]) the information thus lost cannot be recovered by any post recording contrast-enhancing procedure: the loss is irreversible.

Effects of secondary screening

A secondary screening device e.g. a scattered ray grid is inserted into the modulated radiation field behind the object in order to reduce the relative fluence of secondary radiation (cf. STRID). The reduction from the value Φ to the value ψ is defined by the device's selectivity

$$\Sigma = \psi / \Phi \quad (18)$$

however part of the informative (primary) fluence will be lost in the device: changing this fluence from Φ_p to

$$\Phi_p = T_p \Phi_p \quad (19)$$

which defines the device's transmittance for primary radiation T_p .

Since the screening device function by means of anomalously distributed stochastic processes (cf. Appendix) the fluences after secondary screening are still Poisson-distributed. Therefore eqs (15) and (16) still hold true provided that Φ_p is replaced by

Φ_p and ψ by ψ . The signal to noise ratio is then obtained as

$$S_1 = C_p \sqrt{\frac{1}{2} T_p \Phi_p A / (1 + \psi / \Sigma)} \quad (20)$$

Suppose that in a severe case e.g. abdominal radiography $\psi = 7$ (WILSEY 1921). Then with no secondary screening $S_1 \approx S_p / 1.8 \approx 0.55 S_p$. An efficient optimised grid will perform $\Sigma \approx 15$ with $T_p \approx 0.50$ (STRID). Insertion of such a grid will improve the signal to noise ratio to $S_1 \approx 0.58 S_p$ as is seen from eq (20). The improvement may appear marginal. However when the characteristics of the recording devices commonly used are taken into account the efficient grid will come out still more favourable.

Aspects of recording devices

Dynamically limited detectors The expressions (17) and (20) for the signal to noise ratio contain the primary fluence Φ_p . Only if the recording device is free from dynamic limitations can the signal to noise ratio values be compared for the same value of Φ_p . Contrary to this detectors commonly used e.g. film screen combinations or television cameras are dynamically limited. Thus a certain threshold fluence is required for a response to be produced and fluence in excess of a certain saturation value will not produce any further response. The radiation relief with all its modulation detail must thus be contained within the dynamic limits of the recording device.

Consider a detector with the simple characteristics of Fig 3. This is a first-order model of a photographic recording device. In order that a useful image may be produced the total fluence into the device $\Phi_t = \Phi_p + \Phi_s = \Phi_p(1 + \psi)$ must fall within the responsive range of the device. Therefore signal to noise ratio values must be compared at some optimum value Φ_D in the central part of the responsive range. The overall signal to noise deterioration caused by secondary radiation is thus demonstrated by the comparison of S_p relating to $\Phi_p = \Phi_D$ with S_1 relating to $\Phi_p(1 + \psi) = \Phi_D$. Then

$$S_p = C_p \sqrt{\frac{1}{2} \Phi_D A}$$

$$S_1 = C_p \sqrt{\frac{1}{2} \Phi_D A / (1 + \psi) / \sqrt{1 + \psi}}$$

and consequently

$$S_1 / S_p = \frac{1}{1 + \psi} \quad (21)$$

It is seen by the application of eqs (17) and (20) that the case of $\psi \approx 7$ yields $S_1 \approx 0.13 S_p$, whereas the efficient grid gives $S_1 \approx 0.48 S_1$, i.e. a nearly four fold improvement of the signal to noise ratio.

Comparison of different combinations of recording and secondary screening devices Two detectors 1 and 2 requiring for optimum response fluence values Φ_{D1} and Φ_{D2} respectively are considered. They are provided with screening devices (grids) having selectivity values Σ_1 and primary transmittance values T_{p1} ($i=1, 2$). The signal to noise ratio values should then be compared for

$$T_1 \Phi_1 (1 + \psi/\Sigma_1) = \Phi_{D1} \quad (22)$$

For a non ideal recording device it must be taken into account that a certain fraction of the roentgen photons will be lost in the detection process. This is described by the device's detective quantum efficiency (DQE) ε being the probability for a photon to be detected. The detection loss will impair the signal to noise ratio by a factor $\sqrt{\varepsilon}$ (cf DAINLY & SHAW 1974 §5.1).

The mean number of photons detected by the recording device is now

$$\langle \delta V_1 \rangle = C_p \varepsilon T_1 \Phi_1 A \quad (23)$$

and the standard deviation of that number

$$\sigma(\delta V_1) = \sqrt{2\varepsilon T_{p1} \Phi_{D1} A (1 + \psi/\Sigma_1)} \quad (24)$$

which is realised by the appropriate extension of eqs (15) and (16). Combination of eqs (22) and (24) yields the detection signal to noise ratio

$$S_1 = C_p \sqrt{2\varepsilon \Phi_{D1} A / (1 + \psi/\Sigma_1)} \quad (25)$$

Thus

$$S_1/S_2 = \sqrt{\varepsilon_1/\varepsilon_2} \times \sqrt{\Phi_{D1}/\Phi_{D2}} \times \sqrt{(1 + \psi/\Sigma_1)/(1 + \psi/\Sigma_2)} \quad (26)$$

Dose to the object For a proper comparison between radioscopic systems to be performed the dose absorbed by the object must be considered. As far as monoenergetic radiation is concerned the dose is proportional to the incident fluence $D_1 \propto \Phi_{D1} = \Phi_1 / p_p$. From eq (22) then

$$\frac{D_1}{D_2} = \frac{\Phi_1}{\Phi_2} \frac{T_{p1} (1 + \psi/\Sigma_1)}{(1 + \psi/\Sigma_2)} \quad (27)$$

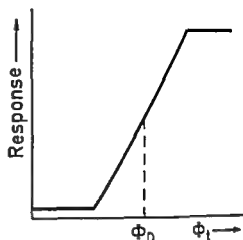


Fig. 3. Idealised characteristic of a dynamically linear recording device.

Comparison at fixed signal to noise ratio Let the sensitivity values of the recording devices be adjusted so that the two systems to be compared yield the same value of S_1 . Then eq (26) can be solved for the expression $(1 + \psi/\Sigma_1)/(1 + \psi/\Sigma_2)$ which in turn is inserted into eq (27) in order to obtain

$$(D_1/D_2)_1 = \sqrt{\varepsilon_1/\varepsilon_2} \times \sqrt{\Phi_{D1}/\Phi_{D2}} \times T_{p1}/T_{p2} \quad (28)$$

where the Φ_{Di} denote the sensitivity values of the recording devices. For grids of good quality $T_{p1} \approx T_{p2}$ and it may as a first approximation be reasonable to assume that $\varepsilon_1 \approx \varepsilon_2$. The dose is then proportional to the square root of the optimum fluence required by the detector (or loosely speaking inversely proportional to the square root of the speed of the detector). It is thus justified to promote the use of film screen combinations of inferior sensitivity provided that secondary screening devices be further improved (REICHMANN & STRID).

Comparison at fixed dose Assume instead that the systems of recording and secondary screening devices are composed in such a way that the detectors yield optimum responses for the same value of incident fluence. The systems can then be compared for the same object dose value F_1 which is solved for $(1 + \psi/\Sigma_1)/(1 + \psi/\Sigma_2)$ which with (25) yields

$$(S_1/S_2)_0 = \sqrt{\varepsilon_1/\varepsilon_2} \times \sqrt{\Phi_{D1}/\Phi_{D2}} \times \sqrt{T_{p1}/T_{p2}} \quad (29)$$

Again it may be assumed that $\varepsilon_1 \approx \varepsilon_2$ and $T_{p1} \approx T_{p2}$. It is then found that the signal to noise ratio for a given dose value is proportional to the square root of the

the detector speed, as far as improved secondary screening will admit, which is consistent with the observations by REICHMANN & STRID

Relation between contrast resolution and spatial resolution

The flux of roentgen photons being a Poisson distributed stochastic process, the signal to noise ratio of the detection of a particular object detail determined by the number of primary photons actually penetrating that detail. Thus for a given value of the photon fluence, the ratio depends on size of the projected area of the detail, as shown in eq. (13). For an object of characteristic lateral extent d , the analysis should then pertain to an elementary image area of size $A = d^2$, whence

$$S_D = C_p d \sqrt{I_0 \Phi_p} \quad (30)$$

and for the general case with secondary radiation and a non ideal recording device

$$S_1 = C_p d \sqrt{I_0 \Phi_D} / (1 + \psi/\Sigma) \quad (31)$$

as seen from eqs. (13) and (25) respectively

For detection of the characteristic object detail to be possible, the signal to noise ratio must exceed some threshold value, i.e. the detectability criterion can be written

$$S_1 \geq S_{\text{threshold}} \quad (32)$$

or, in the case of no secondary radiation and ideal detection, $S_1 = S_D$, and detection is then possible

$$C_p d \geq S_{\text{threshold}} / \sqrt{I_0 \Phi_p} \quad (33)$$

The implication of (33) is obvious in radioscopy under fixed conditions: the limit of contrast resolution varies inversely with the required spatial resolution. Therefore radioscopy of fine object detail, requiring faint contrast, demands high photon fluence and consequently a high dose to the object.

Yet the situation is much more unfavourable than (33) might indicate for the fine structure of the human body extends in three dimensions, and thus details imply low contrast. For a small detail of thickness d embedded in a thick body $C_p \propto d$. If d is taken to be the characteristic detail size in all

three dimensions, then the criterion (33) can be reformulated as

$$d \sqrt{I_0 \Phi_p} \geq \Lambda_1 S_{\text{threshold}} \quad (34)$$

where Λ_1 is a constant. Suppose that an object detail of size d_0 is barely visible at a certain value of Φ_p . Then obviously the detection of a similar detail of size $16d_0$ will require Φ_p to be increased to 16 times the original value.

The discussion of resolution limits can easily be extended to the case with secondary radiation and non ideal detection. By the same considerations as led to eq. (25), the criterion (34) will then read

$$d \sqrt{I_0 \Phi_D} \geq \Lambda_1 S_{\text{threshold}} (1 + \psi/\Sigma) \quad (35)$$

from which the detrimental effect of secondary radiation may be inferred.

From the arguments leading to eq. (27), the dose absorbed by the object is seen to be proportional to the fluence into the recording device

$$D \propto \frac{\Phi_D}{T_p(1 + \psi/\Sigma)} \quad (36)$$

Suppose that the recording speed, described by Φ_D , can be varied at will. Then Φ_D is suitably eliminated from (25) and (36) to yield the detectability criterion as

$$d \sqrt{I_0 T_p D} \geq \Lambda_1 S_{\text{threshold}} \sqrt{1 + \psi/\Sigma}$$

or

$$D \geq K \frac{S_{\text{threshold}}^2 (1 + \psi/\Sigma)}{d^2 I_0 T_p} \quad (37)$$

Λ_1 being another constant. This further emphasises the need for efficient secondary screening for given visibility requirements: the dose will decrease when the relative fluence of secondary radiation admitted to the recording device is reduced, provided that Σ and T_p are not unfavourably affected by the improvement in secondary screening and that a correspondingly faster recording device is used. In more general terms, the detectability criterion (37) demonstrates the interplay of parameters pertaining to secondary screening (Σ , T_p) and to the recording process proper (ϵ). As a corollary, film screen combinations and scattered ray grids should always be considered together as far as recording quality is concerned (REICHMANN & STRID).

Concluding remarks

The consequences of quantum fluctuations in the radiation relief with regard to image quality are elucidated. No experimental evidence has been provided but reference may be made to previous reports (SELIN et coll REICHMANN & STRID REICHMANN & ÅSTRAND).

Although dynamically limited recording devices have been considered the consequences with regard to image noise of the interaction between radiation and photographic type detectors have been left out of the discussion. This aspect will be the subject of a forthcoming report (STRID & REICHMANN 1980).

Appendix

Elements of the theory of discrete stochastic variables

A few fundamental definitions and theorems pertaining to discrete stochastic variables will be presented. For an exhaustive treatment including proofs of the theorems the reader is referred to any standard text on probability theory (e.g. CRAMÉR 1954, TUCKER 1962).

Mean value, variance and standard deviation. Any quantity ξ that can be observed in an experiment is said to be a stochastic variable. Each time the experiment is performed a value for ξ is obtained. A variable that takes only non-negative integer values is termed discrete. Let $P(\xi=k)$ be the probability that the experiment yields $\xi=k$. Then $\sum_{k=0}^{\infty} P(\xi=k) = 1$. For many purposes the stochastic variable is adequately described by its mean value (expectation value)

$$\langle \xi \rangle = \sum_{k=0}^{\infty} k P(\xi=k) \quad (A 1)$$

and its variance

$$\text{var}(\xi) = \sum_{k=0}^{\infty} k^2 P(\xi=k) - \langle \xi \rangle^2 \quad (A 2)$$

It is convenient to define the standard deviation (root mean-square deviation) of the variable as

$$\sigma(\xi) = \sqrt{\text{var}(\xi)} \quad (A 3)$$

Chebyshev's inequality. Probability theory provides an important theorem relating to the expected deviation of the value of ξ observed in a realisation of the experiment from the mean value

$$P\{|\xi - \langle \xi \rangle| > a\} < \text{var}(\xi)/a^2 \quad (A 4)$$

(Chebyshev's inequality) where $P\{|\xi - \langle \xi \rangle| > a\}$ denotes the probability that ξ deviates from $\langle \xi \rangle$ by at least the amount a .

Sample mean value and variance. Suppose that n observations of ξ yield the values $\xi_1, \xi_2, \dots, \xi_n$. Then

$$\bar{\xi} = \frac{1}{n} \sum_{i=1}^n \xi_i \quad (1)$$

and

$$s^2(\xi) = \frac{1}{n-1} \sum_{i=1}^n (\xi_i - \bar{\xi})^2 \quad (2)$$

are the sample mean value and the sample variance, respectively, of this series of observations. According to the central theorem of mathematical statistics, for a large number of observations these quantities converge to the corresponding quantities of the stochastic variable.

$$\lim_{n \rightarrow \infty} \bar{\xi} = \langle \xi \rangle \quad (3)$$

$$\lim_{n \rightarrow \infty} s^2(\xi) = \text{var}(\xi) \quad (4)$$

The product of a stochastic variable by a constant. If ξ is a stochastic variable, then multiplication of ξ by an arbitrary constant a yields a new stochastic variable $a\xi$. From definitions (A 1) and (A 2) it follows that

$$\langle a\xi \rangle = a\langle \xi \rangle \quad (5)$$

and

$$\text{var}(a\xi) = a^2 \text{var}(\xi) \quad (6)$$

The sum or difference between two stochastic variables. Suppose that the sum $\xi + \eta$ of two commensurate stochastic variables ξ and η or the difference between them $\xi - \eta$ is observed in an experiment. Probability theory relates the mean value and the variance of the sum or difference to the corresponding quantities of the constituent variables

$$\langle \xi \pm \eta \rangle = \langle \xi \rangle \pm \langle \eta \rangle \quad (7)$$

$$\text{var}(\xi \pm \eta) = \text{var}(\xi) + \text{var}(\eta) \pm 2 \text{cov}(\xi, \eta) \quad (8)$$

where

$$\text{cov}(\xi, \eta) = \sum_{j=1}^n \sum_{k=1}^m P(\xi=j, \eta=k) (j-k) \quad (9)$$

is the covariance of ξ and η . $P(\xi=j, \eta=k)$ is the probability that in the same observation $\xi=j$ and $\eta=k$ and ξ and η are stochastically independent, i.e. if their observed values are uncorrelated, then $\text{cov}(\xi, \eta) = 0$. eq. (A 12) simplifies into

$$\text{var}(\xi \pm \eta) = \text{var}(\xi) + \text{var}(\eta) \quad (10)$$

The binomial distribution. Consider a flux of N photons incident on a material body of finite area. Suppose that the probability for one photon to be absorbed by the body undisturbed is p . Then the probability that

oton is absorbed or scattered will be $1-p$. Transmis-
ion of photons through a body is one example of experi-
nts that may yield two mutually exclusive results.
Suppose that the experiment is repeated N times (i.e.
photons are sent through the body) and that N_p of
se are transmitted undisturbed. From combinatorics
d probability theory the probability that exactly n
them are transmitted will obtain as

$$P(N_p=n) = \binom{N}{n} p^n (1-p)^{N-n} \quad (A14)$$

here $\binom{N}{n} = N!/(n!(N-n)!)$. This is the frequency ex-
ression of the binomial (Bernoulli) distribution. Further
calculations will show that for this kind of distribution

$$\langle N_p \rangle = N p_p \quad (A15)$$

$$\text{var}(N_p) = N p_p (1-p_p) \quad (A16)$$

The Poisson distribution. The emission of roentgen
photons by the tube target may be regarded as a binomi-
ly distributed stochastic process. Indeed, during a short
interval of time Δt emission may or may not occur and
 Δt is chosen to be sufficiently small the probability of
mission of more than one photon will vanish. Let $\lambda \Delta t$
be the probability for emission to take place during Δt
and let the process be observed a long time $t_{\text{obs}} = N \Delta t$.
Let the experiment be performed N times. During the
time of observation N photons are emitted. In the limit
where $\Delta t \rightarrow 0$ and $N \rightarrow \infty$ in such a way that $N \lambda \Delta t = \text{constant}$
the probability that $N = n$ is calculated as

$$P(N=n) = \exp(-\lambda t_p) (\lambda t_p)^n / n! \quad (A17)$$

which is the frequency function of the Poisson distribu-
on. This distribution thus prevails as a limiting case of
the binomial distribution when the number of experi-
ments is made very large whereas the probability of a
particular result becomes infinitely small. The mean value
and the variance are found to have the same value viz

$$\langle N \rangle = \text{var}(N) = \lambda t_p \quad (A18)$$

Consider again the transmission of radiation through a
material body of finite thickness. Let the flux density be
the area irradiated A and the observation time t_p .
Then on an average the number of incident photons
is $\langle N_0 \rangle = \varphi A t_p$. Emission of roentgen photons being a
Poisson-distributed process the standard deviation of
 N_0 is $\sigma(N_0) = \sqrt{\varphi A t_p}$. Inside the body photons will be
absorbed or scattered leaving the probability p_p for a
photon to be transmitted undisturbed. If the number of
incident photons in an actual experiment were n_0 then
qs (A15) and (A16) would yield for the number of
photons transmitted $\langle N_p \rangle = n_0 p_p$ and $\text{var}(N_p) = n_0 p_p (1-p_p)$.
However the actual number of incident photons is deter-

mined by the stochastic variable N_0 and so the actual
distribution of N_p will prevail as an average for all possible
values of n_0 . Straightforward calculation will yield

$$\langle N_p \rangle = \text{var}(N_p) = N_0 p_p \quad (A19)$$

for a Poisson-distributed flux of incident photons the flux
of transmitted photons is Poisson distributed as well.

The flux of secondary (i.e. scattered and fluorescent)
photons may be treated analogously leading to the same
conclusion.

SUMMARY

Quantum fluctuations in the roentgen radiation relief
are analysed mathematically. The intrinsic signal-to-noise
ratio of the radiation relief for a given object contrast is
proportional to the square root of the number of photons
contributing to the image of a characteristic detail in the
object. In the presence of secondary radiation the signal to
noise ratio is impaired since the fluctuations of
secondary radiation increase the noise of the radiation
relief. By efficient secondary screening the quality of the
relief can be partially recovered. With a dynamically
limited recording device (i.e. a film-screen combination)
increased detection speed in conjunction with improved
secondary screening will either result in unchanged image
quality with the gain of an object-dose reduction or pro-
vide improved imaging at an unchanged dose value. As
regards the relation between contrast resolution and
spatial resolution the dose required to barely demonstrate
an object detail of given geometry and composition is
found to vary inversely with the fourth power of the
linear size of the detail. It is also concluded that re-
cording and secondary screening devices should always
be considered together as far as recording quality is con-
cerned.

ACKNOWLEDGEMENT

This investigation was supported by a grant from the
Swedish Medical Research Council (project No. B79 291
5360).

REFERENCES

- BLACKWELL H. M. Contrast thresholds of human eye
J opt Soc Amer 36 (1946) 624
- CLEAR H. M. SPLETTSOSSER H. R. and SEEMANN
H. E. An experimental study of the mottle produced
by x-ray intensifying screens Amer J Roentgenol
88 (1962) 168
- CRAMER H. The elements of probability theory and some
of its applications. Almqvist & Wiksell/Gebbers Forlag
Stockholm 1954
- DAINTY J. C. and SHAW R. Image science principles
analysis and evaluation of photographic type imaging
processes Academic Press London New York and
San Francisco 1974

- HONDIUS BOIDINGH W The rational choice of an x ray grid *Medicamundi* 6 (1960) 115
- Grids to reduce scattered X rays in medical radiography *Philips Res Rep* (1964) Suppl No 1
- International Commission on Radiation Units and Measurements (ICRU) Radiation quantities and units ICRU Report 19 (1971)
- MORGAN R H The frequency response function—a valuable means of expressing the informational recording capability of diagnostic x ray systems *Amer J Roentgenol* 88 (1962) 175
- Threshold visual perception and its relationship to photon fluctuation and sine wave response *Amer J Roentgenol* 93 (1965) 982
- MOTZ J W and DANCOS M Image information content and patient exposure *Med Phys* 5 (1978) 8
- OOSTERKAMP W J Eliminating scattered radiation in medical X ray photographs *Philips techn Rev* 8 (1946) 183
- REICHMANN S and ÅSTRAND K Influence of radiation dose on image quality *Acta radiol Diagnosis* 20 (1979) 393
- and STRID K G Diagnostically acceptable level of secondary radiation *Acta radiol Diagnosis* 20 (1979) 145
- REISS K H Scattered radiation and characteristic film curve *Radiology* 80 (1963) 663
- RISE A Sensitivity performance of human eye on an absolute scale *J opt Soc Amer* 38 (1948) 196
- ROSSMANN K Spatial fluctuations of x ray quanta in the recording of radiographic mottle *Amer J Roentgenol* 90 (1963) 863
- SCHOBER H und HÖFERT M Die Anwendbarkeit der Optik gebräuchlichen Kontrastübertragungsinformationstheorie auf die Abbildung mit Röntgenstrahlen *Acta radiol Diagnosis* 1 (1963) 1179
- SJELIN K and REICHMANN S High-density films in radiographic films *Acta radiol Diagnosis* 16 (1975) 95
- DIETRICH E and REICHMANN S Influence of secondary radiation on image quality *Acta radiol Diagnosis* 16 (1975) 520
- STRID K G Analysis of secondary screening grids—special reference to grids for abdominal radiography *Acta radiol* (1976) Suppl No 351
- and REICHMANN S Receptor saturation in film-screen systems To be published in *Acta radiol Diagnosis* (1980)
- STURM K E and MORGAN R H Screen intensification systems and their limitations *Amer J Roentgenol* 62 (1949) 617
- TUCKER H G An introduction to probability and mathematical statistics Academic Press New York 1967
- WILSEY B B The intensity of scattered x rays in radiography *Amer J Roentgenol* 8 (1921) 378

NATURAL KILLER ACTIVITY IN PERIPHERAL LYMPHOCYTE POPULATION FOLLOWING LOCAL RADIATION THERAPY

H. BLOMGREN, E. BARAL, F. EDSMYR, L. E. STRENDER
II. PETRINI and J. WASSERMAN

Lymphocytes which express natural cytotoxicity against tumour cells in vitro have recently received much interest because of their possible role in surveillance mechanisms. A positive correlation has been found in experimental animals between natural killer (NK) activity in vitro and capacity to prevent growth of transplanted tumours (HALLER et coll 1977, KASAI et coll 1979, KIESSLING et coll 1975, ARNER et coll 1977). Moreover, mouse strains displaying a high incidence of spontaneous leukemia exhibit low NK activity in vitro and vice versa (LARLING et coll 1975).

Although the role of NK cells in tumour cell surveillance in the human is unknown, it is of interest to examine whether they are affected by treatments which are frequently used to control human malignant tumours. In the present investigation the NK activity in peripheral lymphocytes has been examined after postoperative radiation therapy for breast carcinoma. Previously it has been shown that this treatment induces lymphopenia with an alteration in the proportion of lymphocyte subpopulations (PETRINI et coll 1977), reduces the responses of the lymphocytes to soluble antigen and allogeneic cells (BARAL et coll 1977, BLOMGREN et coll 1977) and reduces the capacity of the cell population to mediate antibody dependent cellular cytotoxicity (ADCC, WASSERMAN et coll 1975).

Material and Methods

The material consisted of 24 women with primary breast carcinoma (32–70 years old), 6 patients with carcinoma of the prostate (62–77 years old) and 7 patients with carcinoma of the urinary bladder (1 female and 6 males with an age range of 61–77 years). The patients with breast carcinoma were considered operable at diagnosis but not the patients with carcinoma of the prostate or of the bladder.

Irradiation techniques. Patients with breast carcinoma were treated with a modified radical mastectomy followed by local irradiation up to a total target dose of 45.0 Gy (4500 rad) as described by IDESTRÖM et coll (1979). Patients with carcinoma of the prostate received local irradiation of the tumour covering the entire small pelvic region but not extending to the juxta regional nodes. The calculated mean tumour dose of 54.0 Gy was given in 8 weeks. Patients with carcinoma of the bladder received irradiation of the bladder with a wide margin including the regional lymph nodes in the 75 per cent isodose curve. The calculated total tumour dose was 64.5 Gy given during 8 weeks with a break of 2 weeks after 32.0 Gy. Patients with carcinoma of the prostate or

bladder were treated with 6 MV roentgen rays using a 3 field technique with 2 wedge filter beams in the front and one open beam from the back.

Blood sampling Blood samples were obtained from the patients with breast carcinoma at four occasions: sample I within one week before postoperative irradiation was started; sample II within one week after completion of irradiation; sample III three to four months after irradiation; and sample IV six to eight months after irradiation. Two blood samples were obtained from the patients with carcinoma of the urinary bladder or the prostate within one week before start of irradiation and with in one week after a total target dose of 54.0 Gy (carcinoma of the prostate) and 64.5 Gy (carcinoma of the urinary bladder).

Separation of lymphocytes Lymphoid cells were separated from heparinized venous blood by centrifugation on Ficoll Isopaque and phagocytic cells were removed magnetically (BLOMGREN 1974). The resultant preparations were used for testing the NK activity of the lymphocyte preparation. T-cells were separated from these purified lymphocyte preparations by rosetting with neuraminidase treated sheep red blood cells (E_a) followed by centrifugation on Ficoll Isopaque (MORETTA et al. 1977).

Rosette techniques Details of these techniques have been presented previously (PETRINI et al. 1979). Lymphocytes possessing membrane receptors for the Fc part of IgG were identified in non separated preparations by their capacity to form rosettes with ox RBC sensitized with rabbit anti ox RBC IgG. Lymphocytes in T-cell enriched fractions possessing Fc receptors for IgG (T_c -cells) or IgM (T_m -cells) were identified by their capacity to form rosettes with ox RBC IgG and IgM respectively. The frequency of T-cells was established by counting cells forming rosettes with E_a .

Cytotoxic test NK activity was measured by incubating lymphocytes for 4 h with ^{51}Cr labelled target cells termed K562 (derived from a human myeloid leukemia) and Chang cells (probably derived from human liver) as described previously (EINHORN et al. 1978). A cytotoxic index was calculated according to the following formula: $(\% \text{ release with lymphocytes} - \text{spontaneous release}) / (100 - \text{spontaneous release})$.

Duplicate tests were performed and variability within the duplicates did not exceed 10 per cent.

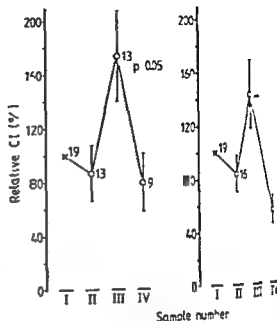


Fig. 1. Relative changes of the ^{51}Cr activity expressed as cytotoxic index (CI) of the peripheral lymphocytes pooled against Chang cells after irradiation for mammary carcinoma. Left diagram shows the results employing a lymphocyte to cell ratio of 40:1 and the right diagram 100:1. Pretreatment values are set as 100 per cent. The absolute pretreatment cytotoxic indices (mean \pm SE) were 0.16 ± 0.04 and 1.1 ± 0.04 respectively. Mean values \pm SE are shown and figures at the symbols indicate the number of patients. A p-value at a symbol indicates the mean differs significantly from the pretreatment value.

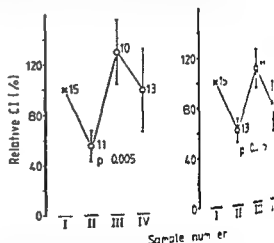


Fig. 2. Same as in Fig. 1 except that K562 cells were used as target cells. The absolute pretreatment cytotoxic indices (mean \pm SE) were 0.54 ± 0.05 using a lymphocyte to target cell ratio of 40:1 and 0.63 ± 0.04 using a ratio of 100:1.

tient. The Student's t test was used for calculating whether the changes were statistically significant.

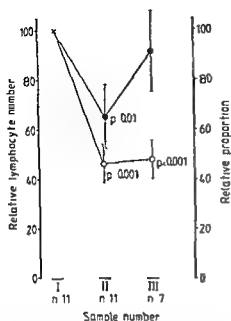


Fig. 3 Relative lymphocyte counts and relative proportions of lymphocytes possessing Fc IgG receptors in non fractionated cell preparations after irradiation of mammary carcinoma. The pretreatment values are set as 100 per cent. The absolute pretreatment lymphocyte count (mean \pm SE) was 2000 ± 00 and the percentage of Fc IgG bearing cells 19 ± 7.4 . Mean values \pm SE are shown. A p value III a symbol indicates that this mean value differs significantly from the pretreatment value. Relative lymphocyte counts (O) relative proportion of lymphocytes possess Fc IgG receptors (●)

Table

percentage of lymphocytes forming rosettes with E_n in non fractionated lymphocyte preparations and the frequency of lymphocytes possessing Fc receptors for IgG (T_G) and IgM (T_M) in E_n cell sedimented fractions. The determinations were made in patients before irradiation and at completion of pelvic irradiation. Mean values \pm SE are shown

	Before irradiation	After irradiation
binding cells	79.9 \pm 7.0	73 \pm 3.5 (9.2 \pm 4.8)
-cells	4.5 \pm 1.6	4.6 \pm 5.0 (11.5 \pm 3.1)
-cells	37.3 \pm 3.5	39.9 \pm 6.6 (110.8 \pm 16.0)

Mean values \pm SE expressed as per cent of the pretreatment values. No statistically significant changes after irradiation

activity of the lymphocyte population exhibited a small but non significant reduction for Chang cells at completion of radiation therapy for breast carcinoma (Fig. 1). This was followed by a significant over shoot three to four months later compared with lymph

phocyte target cell ratio of 50:1. Six to eight months after irradiation (sample 4) NK activity was reduced to the original level or below this level. Most of the patients in this group were also examined for NK activity against K562 cells (Fig. 2). The NK activity against this target cell largely changed similar to that recorded against Chang cells with the exception that the initial reduction was statistically significant and the ensuing increase less marked.

Frequency of Fc receptor bearing lymphocytes following irradiation Since lymphocytes mediating NK activity in the human seem to possess Fc receptors for IgG (COOPER et al. 1977) the proportion of such cells following irradiation was examined. The changes of the lymphocyte counts and the relative proportion of lymphocytes possessing Fc receptors for IgG following irradiation for breast carcinoma appear in Fig. 3. Five of the patients included in this group were also tested for NK activity. The lymphocyte counts were reduced to approximately 45 per cent and the relative proportion of Fc IgG positive cells was reduced to approximately 65 per cent at completion of irradiation. Three to four months after treatment (sample 3) the lymphocyte counts remained at essentially the same level whereas the frequency of Fc IgG positive cells had recovered to approximately 90 per cent of the original value.

Since the NK cell is considered to be a non T-cell by COOPER et al. and since the T-cell population in the blood also contains Fc IgG receptor positive cells (T_G) it was of interest to know whether the decline of the proportion of Fc receptor bearing cells was due to a reduced frequency within the T or the non T lymphocyte compartments. This was analysed in a group of patients who received radiation therapy to the pelvic region and whose lymphocyte counts were reduced to 40 to 60 per cent of the pretreatment values. The frequency of lymphocytes forming E_n rosettes did not change (Table) and the frequency of T_G cells within this cell population was not altered significantly. The proportion of T_M cells also remained essentially constant.

Discussion

The NK activity of the peripheral lymphocyte population was analysed on a cell for-cell basis before and after postoperative radiation therapy for breast carcinoma. Such knowledge may be relevant since data from animal experiments indicate a close

correlation between *in vitro* NK activity and growth of tumour cells *in vivo*.

Somewhat different results were obtained depending on the type of target cell and the lymphocyte target cell ratio. NK activity against K562 cells was significantly reduced shortly after completion of irradiation whereas such a reduction was hardly discernible against Chang cells (Figs 1-2). Three to four months after irradiation the NK activity against Chang cells was above the pretreatment level followed by a decline. No significant overshoot was observed using K562 cells as targets. These results may indicate that the lymphocyte to population mediating NK activity is heterogeneous with respect to radiation sensitivity and mode of recovery after irradiation. Moreover, as shown in Figs 1 and 2, the amplitudes of the changes of the NK activity after irradiation may be dependent on the lymphocyte target cell ratio.

The frequency of lymphocytes possessing Fc receptors for IgG in non fractionated lymphocyte preparations varied similarly to that of the NK activity against K562 cells (Fig. 3). These lymphocytes may be mainly non T-cells as the frequency of Fc IgG receptor bearing lymphocytes within the T-cell population was not significantly changed in patients irradiated for pelvic tumours (Table). Provided that these data can be applied to patients with breast carcinoma, it is possible that the fluctuations of the relative NK activity may at least partly be due to a changed frequency of Fc IgG bearing non T-cells.

It has been observed that local radiation therapy for pulmonary carcinoma augments the cytotoxicity of the peripheral lymphocytes against allogeneic pulmonary carcinoma cells as measured one week after completion of therapy (MANABE *et al.* 1977). Radiation therapy for ovarian carcinoma stage III did not change the cytotoxicity of the peripheral lymphocytes against allogeneic ovarian carcinoma cells in spite of a profound radiation induced lymphopenia. In contrast, radiation therapy for ovarian carcinoma stage I and II caused a significant reduction of cytotoxicity after seven days of treatment which however recovered during continuation of treatment (KOHORN *et al.* 1978). A reduction of cytotoxicity of peripheral lymphocytes against allogeneic urinary bladder carcinoma cells after irradiation was observed by OTTOFF *et al.* (1973).

It should be emphasized that in the investigations mentioned the target cells for cytotoxicity were of the same histologic type as the patient tumours and

probably partially of ADCC type. Therefore, these results may not be strictly comparable with those of the present report. However, several experiments performed in animals have shown that the NK activity is relatively resistant to radiation as measured 24 h of irradiation (HOCHMAN & CUDKOWICZ 1977, KIESSLING *et al.* 1977, OEHLER & HERBERMAN 1978). HOCHMAN *et al.* (1978) observed that NK activity in the spleen did not decline within 14 days after whole body exposure of mice to about 1 Gy (originally 700 R) and recovery started on day 14. They concluded that mature NK cells are relatively radiation resistant whereas their progenitors are relatively sensitive. In contrast, it was concluded by OEHLER & HERBERMAN that rat pre-NK cells are more resistant than mature NK cells since even doses of rats to about 10 Gy (originally 1000 R) did not change the NK cell activity which however was restored by treating animals with polyclonal activators of pre-NK cells.

The fluctuations of the NK activity after radiation therapy as observed in the present investigation may indicate that mature NK cells or their progenitors or both are affected by the treatment. In an attempt to elucidate this question an investigation is started to reveal whether these fluctuations disappear by treating the lymphocytes with interferon which is known to augment NK activity (ERLICH *et al.* 1978, TRINCHIERI & SANTOLI 1978). Additional tests may also reveal whether the NK activity of patient lymphocytes correlate to prognosis of the disease.

SUMMARY

The natural killer (NK) activity of peripheral lymphocytes against ^{51}Cr labelled Chang or K562 cells was measured using a 4 h release assay before and after radiation therapy for mammary carcinoma. NK activity against K562 was significantly reduced at completion of therapy (a total target dose of 45.0 Gy) and recovered 3 to 4 months later. NK activity against Chang cells showed a slight but non-significant decline at completion of therapy followed by an overshoot 3 to 4 months later. The frequency of Fc IgG receptor bearing lymphocytes increased at completion of therapy and largely recovered 3 to 4 months later.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs F. Odmark and Wedin, Mr S. Lindén and Mr K. Gisselberg for excellent technical assistance. This investigation was supported by grants from the King Gustaf V's Jubilee Fund.

REFERENCES

- BARAL E, BLOMGREN H, PETRINI B and WASSERMAN J. Blood lymphocytes in breast cancer patients following radiotherapy and surgery. *Int J Radiat Oncol Biol Phys* 2 (1977) 289.
- BLOMGREN H. Steroid sensitivity of the response of human lymphocytes to phytohemagglutinin and pokeweed mitogen: role of phagocytic cells. *Scand J Immunol* 3 (1974) 655.
- WASSERMAN J, EDENFELT F, BARAL E and PETRINI B. Reduction of responder and stimulator capacities of peripheral lymphoid cells in the mixed lymphocyte culture following external radiotherapy. *Int J Radiat Oncol Biol Phys* 2 (1977) 297.
- JOPLER S M, HIRSHEN D J and FRIEDMAN G J. Spontaneous cell mediated cytotoxicity against Chang cells by nonadherent non thymus-derived Fc receptor bearing lymphocytes. *Cell Immunol* 32 (1977) 135.
- KOHN S, BLOMGREN H and STRANDER H. Interferon and spontaneous cytotoxicity in man. I. Enhancement of the spontaneous cytotoxicity of peripheral lymphocytes by human leukocyte interferon. *Int J Cancer* 22 (1978) 405.
- ALLER O, HANSSON M, KJESLING R and WIGFELT H. Role of nonconventional natural killer cells in resistance against syngeneic tumour cells in vivo. *Nature* 270 (1977) 609.
- KHANNA P S and CUDKOWICZ G. Different sensitivities to hydrocortisone of natural killer cell activity and hybrid resistance to parental marrow grafts. *J Immunol* 119 (1977) 2013.
- , DAUSSET J. Decline of natural killer cell activity in sublethally irradiated mice. *J Nat Cancer Inst* 61 (1978) 265.
- EDENFELT F, PETRINI B, BLOMGREN H, WASSERMAN J, WALLGREN A and BARAL E. Changes of the peripheral lymphocyte population following radiation therapy to extended and limited fields. *Int J Radiat Oncol Biol Phys* 5 (1979) 1761.
- ALLER O, LECLERC J C, MCVAY BOUDREAU L, SHEN R W and CANTOR H. Direct evidence that natural killer cells in nonimmune spleen cell populations prevent tumor growth in vivo. *J Exp Med* 149 (1979) 1760.
- KJESLING R, HOCHMAN P B and HALLER O. Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. *Europ J Immunol* 7 (1977) 655.
- , PETRINI B, KALIN G and WIGFELT H. Genetic variation of in vitro cytolytic activity and in vivo rejection potential of nonimmunized mice against a mouse lymphoma line. *Int J Cancer* 15 (1975) 933.
- KOHN S, EDENFELT F, MITCHELL M S, DWYER J M, KNOWLTON A H and KLEIN ANGERER R. Effect of radiation on cell mediated cytotoxicity and lymphocyte subpopulations in patients with ovarian carcinoma. *Cancer* 41 (1978) 1040.
- MANABE H, YASUNOTO K, OHTA M, TOYOHARA K and NOMOTO K. Effect of anticancer therapy on lymphocyte cytotoxicity in lung cancer patients. *Cancer* 69 (1977) 477.
- MORETTA L, WEBB S R, GROSSI C E, LYDYARD P M and COOPER M D. Functional analysis of two human T-cell subpopulations. Help and suppression of B cell responses by T-cells bearing receptors for IgM or IgG. *J Exp Med* 146 (1977) 184.
- OEHLER J H and HERBERMAN R B. Natural cell mediated cytotoxicity in rats. III. Effects of immunopharmacologic treatments on natural reactivity and on reactivity augmented by polyinosinic polycytidylic acid. *Int J Cancer* 21 (1978) 221.
- O'TOOLE C, PERLMANN P, WIGZELL H, UNSGAARD B and ZETTERLUND C G. Lymphocyte cytotoxicity in bladder cancer. No requirement for thymus-derived effector cells. *Lancet* i (1973) 1085.
- PETRINI B, WASSERMAN J, BLOMGREN H and BARAL E. Blood lymphocyte subpopulations in breast cancer patients following radiotherapy. *Clin exp Immunol* 29 (1977) 36.
- , BIERFELD G, BARAL E and BLOMGREN H. The effect of in vitro irradiation on PHA mediated cytotoxicity and lymphocytes with receptors for the Fc part of Ig. *J Clin Lab Immunol* 2 (1979) 333.
- TRINCHIERI G and SANTOLI D. Antiviral activity induced by culturing lymphocytes with tumor-derived or virus transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J Exp Med* 147 (1978) 1314.
- WARNER N L, WOODRUFF F A and BURTON R C. Inhibition of the growth of lymphoid tumors in syngeneic athymic (nude) mice. *Int J Cancer* 20 (1977) 146.
- WASSERMAN J, MELFEN B, BLOMGREN H, GLAS U and PERLMANN P. Effect of radiotherapy on lymphocyte cytotoxicity in vitro. *Clin exp Immunol* 22 (1975) 230.
- ZARTLING J M, NOLWINSKY R C and BACH F H. Lysis of leukemia cells by spleen cells of normal mice. *Proc Nat Acad Sci USA* 72 (1975) 2780.

EFFECT OF DIFFERENT ^{90}Sr DOSES ON THE MICROSCOPIC
STRUCTURE OF FOETAL MOUSE OVARIES

C. RÖNNBACK

Radioactive isotopes accidentally incorporated into females during pregnancy will consequently so place their foetuses at risk of radiation injury this might disturb the fertility conditions of the contaminated young and even lead to sterility. The radiation sensitivity of the female gonads have therefore been investigated which revealed great variations depending on the stage of development during exposure (PETERS & LEVY 1964, OAKBERG 1968, RÖNNBACK et al. 1971). An internal ^{90}Sr contamination was shown to injure the foetal ovaries more severely than did acute roentgen irradiation with 19 and 76 Gy (20 and 80 R respectively, HENRICSON & NILSSON 1970). The effect on the ovaries of in utero ^{90}Sr treated mouse foetuses depends considerably on the time for contamination during the gestation (RÖNNBACK 1979). The most severe effects were observed when the contamination occurred during the last part of the pregnancy. The injury to foetal ovaries identified at microscopy was therefore correlated to different amounts of ^{90}Sr injected into pregnant mice on the 19th day post coitum. The results are now presented.

Maternal and Method

Female CBA mice aged 70 to 75 days were mated to untreated males of the same strain. The

females were controlled daily and the presence of a vaginal plug indicated the onset of pregnancy (the day for vaginal plug was called day 1). On day 19 post coitum the pregnant females were intravenously injected into a tail vein with a ^{90}Sr solution to give an amount of 11.1 to 370 kBq (0.3–10.0 μCi) per pregnant female in different experiment groups. The treated females were compared with mated untreated control females. The nuclide was administered as carrier free ^{90}Sr nitrate in 1 N nitric acid diluted with physiologic saline to give a strength of about 2.6×10^6 Bq/ml (70 $\mu\text{Ci}/\text{ml}$). When injected the solution was in equilibrium concerning ^{90}Sr and ^{90}Y (The strontium solution was supplied by the Radiochemical Centre, Amersham, England).

The experiment schedule gives the strontium doses administered to the pregnant females in different groups and the number of randomly selected in utero treated females which were killed at day 28, 56 or 84 post partum for analysis of their ovarian content.

Two experiment series with about half a year in between were performed. A control series was run in each part. The higher dose groups 46.3 to 370 kBq (1.25–10 μCi) were run first though the presentation will be given with increasing ^{90}Sr -dose (cf. schedule). The dose of 46.3 kBq was run in both parts to enable a comparison.

Experiment schedule

Dose (kBq) per pregnant female		No. of in utero treated females analysed in different groups	Time for killing (days p.p.)		
			28	46	84
Control	(untreated)	5	5	-	-
11.1	(0.3 μ Ci)	5	5	-	-
22	(0.6)	5	5	5	-
46.3	(1.25)	4	5	5	-
Control	(untreated)	4	4	5	-
46.3	(1.25 μ Ci)	3	5	5	-
92.6	(2.5)	5	5	5	-
185	(5.0)	5	5	5	-
370	(10.0)	5	5	5	-

The asterisks indicate with which control group the 46.3 kBq group in question should be compared.

After killing the females which as far as possible did not originate from the same litter their ovaries were prepared and immediately fixed in Steve's fluid. They were treated according to conventional histologic methods and embedded in Paraplast. The left ovary from every female was serially sectioned with a thickness of 5 μ m and every 10th section was brought to microscopic analysis including counting oocytes and follicles in different stages of development.

The cell material was divided into 7 classes as in previous investigations: oocytes of type I without any surrounding granulosa cells; type II and III oocytes with an increasing number of such cells (but not forming a complete layer); primary follicles with one complete layer of granulosa cells and growing follicles with several layers but with no antrum formation; early antrum follicles with the antrum initiated and finally Graafian follicles with a complete antrum formation in the follicular epithelium. Two degenerating stages were also counted: atretic follicles and corpora atretica. The former with lytic and pyknotic cells in the follicular layers and often with a shrunken oocyte and the latter being the final stage of atresia in growing and Graafian follicles.

The observed number of cells in these nine stages was adjusted to give the total number in the ovary by means of a correction formula (ABFRÖMME 1946). These corrected figures from the strontium groups were compared with those of corresponding controls to give an estimation of the effect in terms of a reduced number of cells caused by the nuclide.

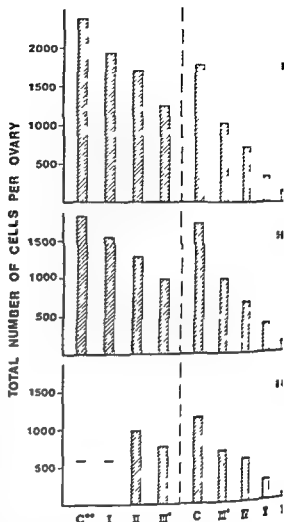


Fig. 1. Total number of remaining cells in ovaries from females treated with ^{90}Sr on day 19 or their foetal ovaries. The females were examined when aged 28, 46 or 84 days. (A) untreated control (second part); (B) 11.1 kBq; (C) 22, 46.3 kBq per pregnant female respectively. (C) = control (first part); (I) 46.3 kBq; (II) 92.6 kBq; (III) 185 kBq; (IV) 370 kBq per pregnant female respectively. The asterisks indicate with which control group the 46.3 kBq group in question should be compared.

Results

The total number of cells calculated on the left ovary is given in Fig. 1. It is very comparable between strontium treated groups and corresponding control groups and corresponds to a statistically significant difference in the number of cells. Fig. 2 presents in the same way the number of oocytes and follicles in the different stages of development in the two control groups as well as in the strontium treated groups. The differences are between 11.1 and 370 kBq per animal (two groups). The graphs also show the variation in the number of cells by age as the figures are given from animals killed 28, 46 or 84 days post partum. (In the second part no analyses were available from animal killed 84 days in the control and 11.1 kBq groups.)

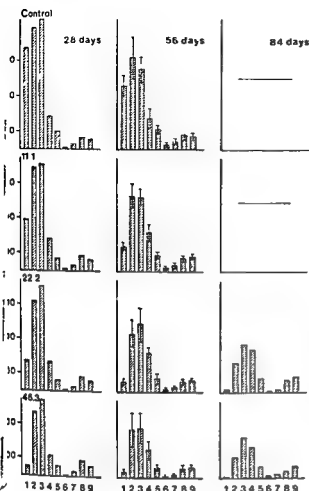
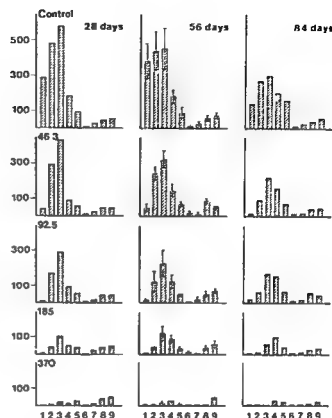


Fig. 1. The number of cells in different stages of development (1-9) for ^{90}Sr doses between 11.1 kBq and 370 kBq given on day 19 post coitum. Stage 1 oocyte type I, 2 oocyte type II, 3 oocyte type III, 4 primary follicles, 5 growing follicles, 6 early antral follicles, 7 Graafian follicles, 8 atretic follicles, 9 corpora atretica.

The frequency of oocytes I-III at day 84 was about half of that at day 28 irrespective whether the animals were strontium treated or not. The frequencies of cells in the following stages were not remarkably altered neither in the control groups nor in the strontium treated ones.

The naked oocyte (stage I) has previously been found to be very sensitive to radiation. Also in the present investigation appeared a strongly significant decrease of the number (to 50% of the control value) even in the lowest dose group where 11.1 kBq ^{90}Sr was administered to the investigated animals mother during her pregnancy. This observation was made both at day 28 and day 56.

When the dose increased to 22.2 kBq the effect was much more marked and also the stages of oocyte II and III were affected. After 46.3 kBq only about 10 per cent of type I oocytes and between 50 and 60 per cent of oocytes II and III remained com-



type III 4 primary follicles 5 growing follicles 6 early antral follicles 7 Graafian follicles 8 atretic follicles 9 corpora atretica

pared with corresponding control figures. Statistically significant decreases in all three stages as well as in the number of Graafian follicles. Still higher doses caused stronger effects and after 370 kBq naked oocytes and practically also type II oocytes were eliminated and only about 3 per cent of type III oocytes remained. Also the other stages except for corpora atretica (stage 9) were severely affected.

Within the dose range 11.1 to 185 kBq ^{90}Sr the primary follicles (stage 4) showed a tendency to increase in number by age, a tendency not found in untreated animals.

In the two lowest dose groups the number of primary follicles at day 56 had increased compared with the controls (though not statistically significant). After higher doses a reduced number of primary follicles was observed in accordance with that of the level of other cell stages.

The frequencies of cells in the nine stages were

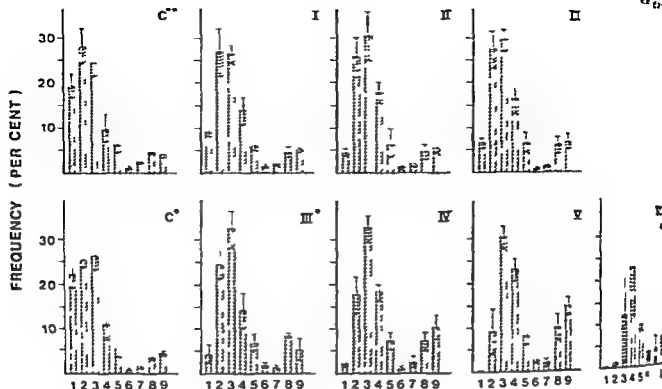


Fig. 3. The frequencies of cells in the different stages (cf. Fig. 2) calculated as per cent of the total number of cells per ovary for every individual. Mean \pm deviation at a 5% risk level.

calculated as per cent of the total number per ovary for every individual in order to eliminate differences depending on variations between females in their total number of oocytes and follicles. These figures gave the means and deviations at a 5 per cent risk level at day 86 (Fig. 3). At day 28 and day 84 the animals showed a similar trend when compared with controls of corresponding age. The decrease in the number of naked oocytes is evident also when calculated in that way. That decrease is the only major change in the two lowest dose groups, but a tendency to an increased fraction of early antrum follicles might also be observed. With doses of ≥ 6.4 kBq ^{90}Sr and higher the fraction of young oocytes still decreases. The fraction of primary follicles (stage 4) seems to increase by dose. In the highest dose groups there seems to be an increase of the fraction of degenerating stages (8 and 9) depending on that way of calculation.

The total number of cells per ovary at the three examined ages is given in Table 1. In Table 2 these figures have been recalculated to give the frequency of cells in the strontium groups expressed as per cent of those in corresponding control groups. The percental figures were then plotted against dose (i.e. the amount of strontium) in a semilogarithmic

diagram (Fig. 4). At day 84 no figures were available for 11.1 and 22.2 kBq ^{90}Sr and therefore a line has been extrapolated for these doses. A significant negative correlation is evident between the magnitude of the remaining cell fraction in females in utero and log dose when the ^{90}Sr was administered on the gestational day 19.

Discussion

Investigations on foetal ovaries have shown a high sensitivity to ionizing radiation resulting from the injury to (and thus elimination of) oocytes. The loss of cells is according to the present knowledge looked upon as important and might influence the fertility conditions and the reproductive capacity of such in utero females.

It has been shown (RÖNNBACK 1979) that when the contamination occurs during the foetal development is a very important factor in determining the degree of injury to the foetal ovaries. When mice were internally contaminated with ^{90}Sr on day 15 post coitum the injury expressed as a reduction of the number of oocytes and (

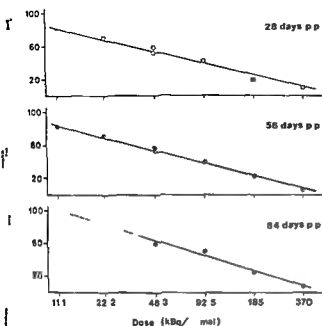


Fig. 4. Remaining fraction of cells in ^{90}Sr treated ovaries (in per cent of respective controls) versus log dose.

Table 1

Total number of cells per ovary (mean \pm SE)

	Days post partum		
	28	56	84
control	2 479.5 \pm 62.3	1 836.0 \pm 65.7	—
11.1 kBq	1 935.4 \pm 47.9	1 531.1 \pm 40.8	—
22.2	1 707.6 \pm 48.7	1 295.7 \pm 51.1	995.6 \pm 44.9
44.3	1 254.7 \pm 49.6	997.6 \pm 59.2	780.1 \pm 40.9
control	1 777.9 \pm 35.2	1 723.1 \pm 76.8	1 145.4 \pm 36.5
63	1 033.4 \pm 45.6	991.8 \pm 36.1	685.2 \pm 36.4
75	735.7 \pm 28.4	685.4 \pm 45.4	586.3 \pm 22.6
88	333.3 \pm 25.5	389.7 \pm 77.7	780.8 \pm 21.6
70	172.7 \pm 8.9	134.1 \pm 17.1	95.7 \pm 11.3

The asterisks indicate with which control group the 46.3 kBq group question should be compared.

...aining in the adult females was much stronger than after a contamination during any earlier part of the pregnancy. The most severe effect was found when the contamination occurred on day 19 with only about 5 per cent of the cells remaining. It was proposed that this strong effect mostly was developed by ^{90}Sr incorporated into the foetal skeleton where it could act as a permanent radiation source during the radiation sensitive period following birth.

NILSSON & HENRICSON (1969) used 740 kBq (20

Table 2

The frequencies of cells in different histological groups given as per cent of respective control values

	Days post partum		
	28	56	84
11.1 kBq	79.7	83.4	—
22.2	70.3	70.6	—
46.3	51.6	54.1	—
46.3	58.3	57.6	59.8
92.5	41.5	39.8	51.7
185	18.8	22.6	74.5
370	9.7	7.8	8.4

μCi) ^{90}Sr per pregnant female. RÖNNBACK (1979) 370 kBq when he analysed the correlation between the time for contamination and the injury to the ovaries and it was fully evident that these strontium doses caused serious reductions of the number of oocytes and follicles.

The purpose of the present investigation was to find out how small an amount of ^{90}Sr administered on day 19 of foetal development that could give a microscopically notable reduction of the number of oocytes and follicles in the foetal ovaries.

The experiment had to be performed in two separate parts: the one with higher strontium doses being run first. A comparison of the figures in Table 1 from females at day 28 indicates that a (significant) difference exists both between the two control groups and between the 46.3 kBq groups at that age. However, this difference had more or less disappeared at day 56. Nor was there any significant difference at day 84 between the two 46.3 kBq groups.

These discrepancies are not considered having seriously influenced the further evaluation of the experimental data as presented in Fig. 4. The remaining fraction of cells in the ^{90}Sr treated ovaries expressed as per cent of corresponding control figures were plotted in semilogarithmic diagrams versus the dose (i.e. the amount of ^{90}Sr injected into the pregnant females). The plotted lines reveal that the injury (reduced number of cells) is strongly correlated to log dose. It is also evident that the rate of injury is almost identical for the ages examined as the slope of the three lines is practically identical. Therefore, it might be suggested that the reduction seems to occur at the same rate independent of the initial number of cells.

As stated previously (RÖNNBACK et al. 1979)

Table 3

Retention of ^{90}Sr and ^{90}Y in ovaries of 10-day-old females and their mothers when injected on day 19 of gestation. The animals were in equilibrium with ^{90}Y 3.0 kBq ^{90}Sr injected per pregnant female. Data from the mothers after a given day are given in the same line

Offspring					Mothers				
No. of offspring	Ovaries	Total weight of ovaries (mg)	Per cent of injected amount per kg tissue		Ratio $^{90}\text{Y}/^{90}\text{Sr}$	Total weight of ovaries (mg)	Per cent of injected amount per kg tissue		Ratio $^{90}\text{Y}/^{90}\text{Sr}$
1	2	3	^{90}Sr	^{90}Y		4	^{90}Sr	^{90}Y	
1	4	7	139	45	0.33	13.0	8.3	47	6.8
2	4	8	18.1	57	0.58	11.6	3.7	51	11.4
3	-	-	6.9	4	0.56	17.7	20.3	41	0
4	4	11	19	8	0.41	26.2	9.9	2	2
1	11	13	17.4	50	0.53	14.8	44.0	86	0
8	4	4	5	57	1.00	17.9	3.8	3.8	10
7	3	4	7.8	31	0.57	14.5	4.5	15	14
Mean		1.46	83.9	45.9	0.47	8.26	13.5	39.4	4.4
SE		± 0.70	± 34.16	± 19.1	± 0.08	± 0.91	± 5.54	± 10.67	± 1.66
		(mean weight of one ovary)				(mean weight of one ovary)			

BACK 1979) young oocytes and especially those in the first stage of development without any surrounding granulosa cells have an extremely high radiation sensitivity. The present investigation reveals that even 11.1 kBq ^{90}Sr given to the pregnant females significantly reduced the number of oocytes in the foetal ovaries.

The real numbers of remaining oocytes and follicles in the different dose groups are given in Fig. 2. A similar but somewhat different way to present the effect of the nuclide appears in Fig. 3 where the Y axis indicates the frequency of cells (given as per cent) in different development stages calculated on the total number of cells per female. These histograms underline the fact that especially the frequency of naked oocytes are affected by very low doses of the nuclide. On the other hand the proportions of the next two stages of developing oocytes seem not to change until the strontium doses reach 92.5 kBq (2.5 μCi) and higher. The increased proportion of degeneration cell stages (8 and 9) at higher doses is a consequence of the presentation technique (cf. Fig. 2 b) where the actual numbers of these stages decrease).

It has not been possible to determine the uptake of ^{90}Sr and ^{90}Y in the foetal ovaries with available technique. APPELREY et al. (1966) have shown

by autoradiography that the ovaries and especially the follicle walls of non pregnant mice have a high accumulation capacity with regard to ^{90}Y . This observation has been corroborated by WALLIN & MÜLLER (1975) who also found that the uptake of ^{90}Y in soft tissues was much higher than that of ^{90}Sr when the latter nuclide was administered in equilibrium with ^{90}Y to adult female mice. In addition we found that the uptake of ^{90}Sr in the mouse ovaries was significantly higher than that in other soft tissues.

In an attempt to achieve a crude indication of the uptake and retention of ^{90}Sr and ^{90}Y in the ovaries of the foetuses and their mothers 370 kBq of ^{90}Sr in equilibrium with ^{90}Y was injected in seven pregnant mice on day 19 of gestation. The mothers and their offspring were killed when the latter were 10 days old. The ovaries were prepared and analysed with respect to their ^{90}Sr and ^{90}Y content using the methods of WALLIN & MÜLLER (1975). The results are given in Table 3 where the data for each litter are presented on the same line. The data from the mother are correspondingly presented in Table 3 for non mated females are presented in Table 4.

The activity concentrations were considerably higher in the mothers as well as in their offspring than in the non mated mice. Due to the high variability in the data it is impossible to give a precise

Table 4

Retention of ^{90}Sr and ^{90}Y in ovaries of non mated females 10 days after the injection of a ^{90}Sr solution in equilibrium with ^{90}Y 370 kBq ^{90}Sr injected per female

	Total weight of ovaries (m_g)	Ratio $^{90}\text{Y}/^{90}\text{Sr}$	Per cent of injected amount per kg tissue	
			^{90}Sr	^{90}Y
	3.0	2.7	9.5	26
	7.6	1.8	13.1	2.4
	7	2.1	5.4	11
	21.6	2.8	7.9	22
	70.0	1.7	8.9	7.4
Mean \pm E	10.90 \pm 0.27 (mean weight of one ovary)	2.4 \pm 0.70	9.0 \pm 2.74	7.4 \pm 2.68

differences between the concentrations of activity in the maternal ovaries and those in the offspring as well as between mothers and non mated females. There is a possible significance ($p < 0.05$) in the ovarian $^{90}\text{Y}/^{90}\text{Sr}$ ratios between the litters and their mothers and a corresponding clear significance ($p < 0.001$) between the young and the non mated females indicating a higher uptake of ^{90}Sr than of ^{90}Y in the foetal ovary compared with that in the adult mouse. The high uptake and the long lasting activity in the foetal ovary imply indubitably a considerable contribution to the radiation dose to the oocytes.

In addition the mineralization of the foetal skeleton causes an incorporation of great amounts of ^{90}Sr and ^{90}Y in growing bones. The radiation from these deposits to the ovaries is certainly not negligible. The dose contributions from deposits in the maternal skeleton is—according to autoradiographic evidence—probably of less importance.

The figures in Table 3 indicate that the radiation dose of the foetal ovary is high but even if contributions from ^{90}Sr and ^{90}Y deposits in the foetal skeleton are taken into account it is not likely that the doses to the foetal oocytes are much higher than the doses to the oocytes in the adult mouse when injected with the same amount of ^{90}Sr . This conclusion is all the more probable as the high activity concentration in the mature follicles has no correspondence in the foetal ovary.

The marked effects on the oocytes in mice exposed in utero to ^{90}Sr and ^{90}Y must therefore in all essentials be explained by the high radiation sensitivity of the young oocytes especially as they have been exposed during stages of extreme susceptibility i.e. just before the onset of and partly during the period of arrested development (the diplotene stage) around the birth and the first two weeks of age (RUGH & WOHLFROHN 1964; RONNBACK 1967).

The estimation of the retention of the nuclide in the juvenile ovaries at day 10 reveals great variations between individuals. Such variations were also found in the ovaries from the mothers as distinguished from the results in non mated females. Whether these variations reflect corresponding disparity in the radiation doses seems doubtful since the microscopic findings do not show differences of the same order of magnitude between individuals in the same dose group. This might be considered as a dominance of the radiation from the foetal skeleton to the ovaries being so much higher than the contributions from nuclides deposited in the ovarian tissues.

Though there is a statistically significant loss of young oocytes in mice treated in utero with very low doses of ^{90}Sr a pool of functional follicles seems to remain. Preliminary results in a fertility experiment in mice with doses similar to those given in the present one indicate that this pool during certain circumstances might be large enough to permit a nearly unchanged reproductive capacity in the adult females. However it must also be remembered that the number of remaining oocytes and follicles are given per one ovary and thus must be doubled to be valid for the individual. Samples taken at random from the present material have not given evidence for differences in the number of oocytes and follicles between the ovaries in the same individual as was also found in monkeys by ANDERSEN *et al.* (1977).

SUMMARY

Pregnant CBA mice were injected with different doses of ^{90}Sr on the 19th day post coitum. The doses ranged between 11.1 and 370 kBq (0.3–10.0 μCi) per animal. The ovaries of the in utero treated females were analysed when the animals were 28, 56 or 84 days old respectively. The effect of the nuclide was expressed as a reduction of the number (frequency) of cells in different stages of ovarian development in a comparison between ^{90}Sr treated animals and untreated controls. The injury (reduced number of cells) was strongly correlated to log dose. The most radi-

tion sensitive stage (the naked oocytes) was clearly affected even at the lowest dose level. The different ovarian $^{89}\text{Y}/^{86}\text{Sr}$ ratios in litters and their mothers are discussed.

ACKNOWLEDGEMENT

The author is greatly indebted to Mrs V. Nilsson for her excellent technical assistance.

REFERENCES

- ALPEROWITZ M. Estimation of nuclear population from microtome sections. *Anat. Rec.* 94 (1946) 239.
- A. DIPSEN A. C., HENDRICKX A. G. and MOMINI M. H. Fractionated λ irradiation damage to developing ovaries in the Bonnet monkey (*Macaca radiata*). *Radiat. Res.* 71 (1977) 374.
- APPELBERG I., NILSSON A. and LIEPPE S. Distribution of yttrium 91 in mice studied by whole body autoradiography. *Acta radiol. Ther. Phys. Biol.* 4 (1966) 41.
- HENDRICKX B. and NILSSON A. Roentgen ray effects on the ovaries of foetal mice. *Acta radiol. Ther. Phys. Biol.* 9 (1970) 443.
- NILSSON A. and HENDRICKX B. The effect of ^{89}Y on the ovaries of the foetal mouse. In: *Proc. Symp. Biology of the Fetal and Juvenile Mammal*. Richland Wash. USA 1979.
- OAKRIDGE F. T. Mammalian gamete response to comparisons in radiation response of the female. Effects of radiation on meiotic systems p. 111. Vienna 1968.
- PIETZ H. and LEVY E. Effect of irradiation on the mouse ovary. A quantitative study of oocyte sensitivity. *J. reprod. Fert.* 7 (1964) 37.
- RÖNNBÄCK C. Effect on fertility of continuous λ irradiation during the suckling period in mice. *Acta radiol. Ther. Phys. Biol.* 6 (1967) 93.
- Effect of ^{89}Sr on ovaries of foetal mice. Time for administration during pregnancy. *Acta Oncology* 18 (1979) 225.
- HENDRICKX B. and NILSSON A. Effect of different doses of ^{89}Sr on the ovaries of the foetal mouse. *Acta radiol.* (1971) Suppl. No. 310 p. 199.
- RUETHER and WOLFF (M.M.) X radiation sensitivity of the premature foetal mouse. *Atomkernenergie* 14 511.
- WALLINDER G. and MULLER W. A. Conception of ^{89}Y and ^{86}Sr in various organs in the foetal mouse. *Report C 400/21 A3* (1975).

CHROMOSOME COUNTS OF ^{90}Sr -INDUCED OSTEOSARCOMAS IN MICE

II Variation of the chromosome counts of slow and fast growing tumours in hyper- and nonhyperimmunized hosts

H. BERGMAN

Osteosarcomas induced by ^{90}Sr have been examined for different characteristics previously (NILSSON 1962, 1966, 1969; NILSSON & RÖNNBACK 1973). Data indicating that such osteosarcomas possess specific transplantation antigens were presented by NILSSON *et al.* (1972). It was then observed that the incidence of progressively growing tumours was significantly greater in whole body irradiated (4 Gy, 400 R) than in unirradiated mice. Furthermore, heavy irradiation (150 Gy, 15 000 R) of tumour cells before transplantation decreased the frequency of progressively growing tumours in comparison to treatment with heavily irradiated normal tissues.

Chromosome number variation in serially transplanted ^{90}Sr induced bone tumours in CBA mice has been reported by BERGMAN & NILSSON (Part I). Previous data have shown that when relating the outgrowth period of primary tumours to the chromosome pattern (KATO 1968; MARK 1969; LITELMAN 1971) fast growing tumours exhibited a normal chromosome distribution while tumours with deviating chromosome numbers were slower growing. Therefore a primary aim of the present investigation was to test whether a connection may exist between the outgrowth period and the variation in chromosome pattern of transplanted ^{90}Sr induced tumours. For this purpose separate serial transplantation was performed from both a fast and a slow growing tumour of one and the same transfer generation. Furthermore, in order to obtain a prolonged

outgrowth period transplantation was also carried out to recipients with a changed immunologic response so called hyperimmunized mice. Besides outgrowth period, tumour size and the presence of metacentric chromosomes were recorded. Histologic characterization of the primary tumour and transplanted tumours was also performed.

Material and Methods

Transplantation procedures Transplantation was always performed to the same sex of the highly inbred strain of CBA mice in order to minimize factors that may favour changes. Therefore only 60 ± 5 days old male mice were used. From a mouse which had received an injection of 14.8 kBq (0.4 μCi) $^{90}\text{Sr}/\text{g}$ body weight a radiation induced osteosarcoma of predominantly fibroblastic type appeared after 403 days. By serial transplantation *in vivo* from the primary tumour (NILSSON & ULLBERG 1962) the numerical chromosome progression of 34 succeeding transfer generations was analysed as B series which had been established previously (Part I). Thus from the second transfer generation (B²) separate serial transplantation (Fig. 1) was performed from a tumour with an outgrowth period of 21 days (transfer series B) and from a slower growing tumour with an outgrowth period of 44 days (transfer series b). Transplantation was per-

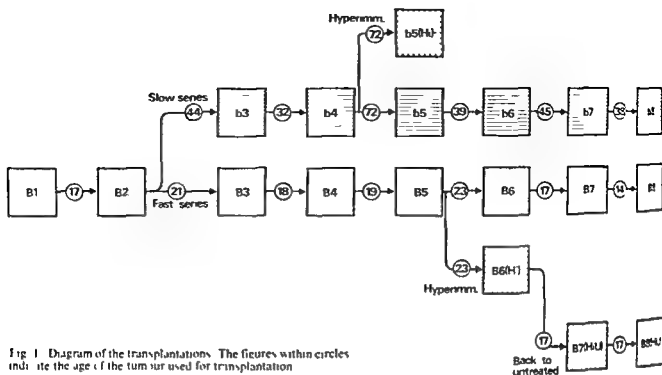


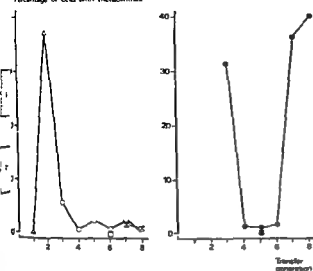
Fig. 1. Diagram of the transplantations. The figures within circles indicate the age of the tumor used for transplantation.

Table 1

Table 1. Distribution of chromosome numbers in B1 (fast series), b3 (slow series), b5 (H) and B6 (H) = Transplantation performed from hyperimmune sera, b7 (H) = Transplantation performed from hyperimmune sera, B5 (H) = Transplantation performed from untreated recipients, B8 (H) = Transplantation performed from untreated recipients.

Transfer generation	No. of tumors examined	No. of cells counted	Chromosome number															
			39	40	41	43	47	48	49	50	51	52	53	54	55	56	57	58
B1	5	175		65.6	1.6												16	18
B2	5	1.5		25.6						0.8						4	7	14
B3	5	1.5	2.4	4									0.8			1	1	14
B4	5	1.5		51.2									0.8			4.8	4.8	1
B5	5	1.5		33.6										0.8	0.8	4.8	0.8	
B6	5	1.5		73.6														13
B7	5	1.5		39.2														
B8	5	1.5		45													0.8	
B9	5	48		33.3													4	4
B10	4	100		57.0													10	18
B11	5	1.5		74.0													5.8	
B12	5	1.5		3.2						0.8		0.8				0.8	0.8	14
B13	5	1.5	1.6	36.0		0.8				0.8		4.6	7.2	36.0	4.0	5.6	4	
B14	5	1.5		16.0	0.8	7.4	0.8	1.6	2.4	4.0	3.2	6.4	13.6	0.8	4.0	4.8	4.8	4.8
B15 (H)	5	1.5		16.0										0.8	4.0	4.8	4.8	4.8
B16 (H)	5	1.5	0.8	36.8												0.8	4	4.8
B17 (H)	5	1.5	0.8	46.0														4.8
B18 (H)	5	1.5		66.4									0.8			0.8	0.8	

Percentage of cells with metacentrics



Percentage of cells with one or more metacentric chromosomes. Δ Fast series transfer generations B1-B8. \circ Fast series transfer generations B3-B8. \square Transfer generation B6 (Hi). Σ Transfer generations B7 (HiU) and B8 (HiU). \bullet Slow series transfer generations b3-b5. \blacksquare Transfer generation b5 (Hi).

formed from transfer generation b4 also to hyperimmunized recipients b5 (Hi) (Fig. 1). In the same way transplantation was carried out from transfer generation B5 to hyperimmunized mice B6 (Hi). From this generation two succeeding transplantations were performed but now to untreated recipients B7 (HiU) and B8 (HiU).

Hyperimmunization assays Suspensions from tumours of transfer generation b3 and B4 respectively were separately prepared by pressing the freshly excised tissue through a stainless wire gauze into 2 ml saline solution. The suspension was cooled with ice cubes and irradiated with 140 Gy (15000 R). Doses of 0.2 ml concentrated cell suspension were injected subcutaneously in both flanks of the mice. The injections were given three times at weekly intervals. One week after the last transfer of heavily irradiated cell material, separate transplantation was performed from a tumour of transfer generation b4 to both hyperimmunized (b5 (Hi)) and untreated mice (b5) and also from a tumour of transfer generation B5 to hyperimmunized (B6 (Hi)) and to untreated (B6) (Fig. 1). It was not considered necessary to estimate the number of cells transplanted since the main purpose was to analyse the chromosome pattern and not the transplantation antigenicity. Extreme care was taken to choose tumour tissue from the same area and of a similar size as when transplanting to untreated hosts.

Chromosome analysis The chromosome preparations were obtained according to the air-dry technique described in Part I. At the most 5 successful tumours per transfer generation were examined and if possible 25 cells per tumour were analysed altogether 2148 cells. Cells suitable for chromosome counting were selected at magnification 200 \times and analysed at 1000 \times . Only the percentile chromosome distribution per transfer generation is presented. No structural analysis or karyotyping was performed primarily due to limited possibilities with the conventional staining method used.

Histologic examination Sections of the tumour were fixed in Steve's solution, embedded and stained according to the van Gieson method or with haematoxylin-eosin. The definition of tumour classification has been presented previously (NILSSON & RÖNNBACK).

Results

Registration of chromosome abnormalities The registration of abnormalities was limited to numeri-

Table 1 (cont.)

	60	61	62	63	64	65	80
37	74	40	56	88	48	08	
36	23	104	3	16	16		
64	504	48	16	16			
16	48	32	56	96	37	16	
08	80	48	200	184	56	16	
		08	10	128	08		
4	80	168	16	88	74		
40	88	178	144	40			
47	1	167	83	1			
0							
08							
08							
	16						08
16	08			08			
16	48	160	00	100	3		
08	4	104	23	32	4		
08	3	88	136	40	16		

Table 2

The percentile chromosome distribution often found between the tumours of a transfer generation is illustrated by the pattern obtained from the tumours of three B generations. 25 cells per tumour were analysed

Transfer generation	Tumour No.	Growth period (days)	Size (cm ³)	Chromosome number															
				39	40	41	53	54	55	56	57	58	59	60	61	62	63	64	65
B1	11	16	0.78	19	2							1	1						
	1	17	0.96	14							1			3		3			
	13	0	0.59	19											1		4		
	14	1	0.91	18												3	1	3	
	15	1	0.72	11							1	1	3		2	1	4	1	
B2	21	1	1.40	2	5						1	1	1	12	2		1		
	3	1	0.84		1						3	3	3	15					
	33	18	1.14		1		1						1	21	1				
	34	18	0.88		4							4	3	11	2		1		
	35	0	0.40	1	17										4	1	2		
B6	61	1	0.76		25														
	6	11	1.26		18											1	3	3	
	6	14	1.31		2												1		
	64	14	0.90		15												3	6	1
	65	17	0.90		12												7	6	

cal chromosome deviations and the occurrence of metacentric chromosomes (Part I). Thus numerous occasionally appearing structural rearrangements were not recorded.

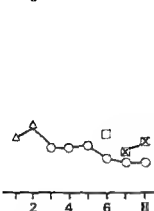
The percentile chromosome distribution per transfer generation is recorded in Table 1. The variation in chromosome numbers between the tumours of a transfer generation is illustrated in Table 2. A diagram of the transplantation appears in Fig. 1. The percentage of cells with one or more metacentrics per generation is presented in Fig. 2. The mean size and the mean growth period of the tumours per generation are presented in Figs. 3 and 4. Finally, Figs. 5 and 6 show a 58-chromosome cell and a 62-chromosome cell respectively.

Chromosome distribution. In the first transfer generation (B1) a predominating number of 40-chromosome cells (65.6%) and a wide distribution within the triploid region was observed (Part I). In the second generation (B2) the percentage of 40-chromosome cells had declined to 25.6 per cent while instead an increased number of cells was found within the triploid region. From this generation transplantation was carried out both from a fast and from a slow growing tumour. By separate serial transplantation the two parallel transfer series (B and b) were established (Fig. 1). When comparing the chromosome distribution of transfer generations

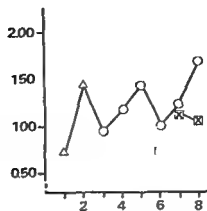
B3 and b3 generation B3 showed 50.4 per cent 40-chromosome cells (range 57 to 63 generations, range of 55 to 63 and flat peaks at 57 and 61 (29.16.7% respectively). In contrast to generation b3 only 2.1 per cent 60-chromosome cells were found. The percentage of 40-chromosome cells was 50.4 per cent (B3) and 33.3 per cent (b3). It should be noticed that only 48 cells were analysed from the b generation. The subsequent B generations (B4-B8) showed a rather high percentage of 40-chromosome cells (33.6-73.6%). Within the triploid region the 61 to 63-chromosome cells were most frequently appearing types. In transfer generations b4 to b8 a different chromosomal pattern was observed in the sense that the only generation with a preponderance of 40-chromosome cells was b4 (59.0%). Also in contrast to the B series the b-series was characterized within the triploid region by cells with continuously lower chromosome numbers. Peaks were found within the 54 to 57 region.

As from transfer generation b4 transplantation was performed also to hyperimmunized mice. The generation designated b5 (H) was obtained by comparing this generation with the control generation untreated generation b5 rather similar chromosomal pattern was found but with the difference that the b-generation had peaks at 55 and 56 (11.4 and 44.0% respectively) and 24.0 per cent 40-chromosome cells.

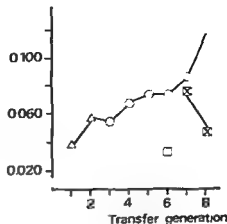
Mean growth period



Mean size



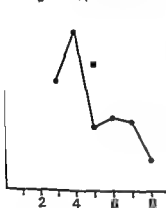
Mean growth per day



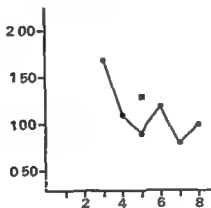
Mean growth period (days) mean size (cm³) and mean growth per day (cm) of the B tumours per generation. Δ Fast transfer generations B1-B5 \circ Fast series transfer genera-

tions B3-B8 \square Transfer generation B6 (Hi) \otimes Transfer generations B7 (HiU) and B8 (HiU)

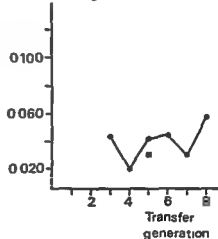
Mean growth period



Mean size



Mean growth per day



Mean growth period (days) mean size (cm³) and mean growth per day (cm) of the b-tumours per generation. \bullet Slow transfer generations b1-b8 \blacksquare Transfer generation b5 (Hi)

cells while generation b5 (Hi) displayed peaks 6 and 57 (24.8 and 41.6% respectively) and 16 cent 40-chromosome cells. Transplantation was performed from generation B5 to hyperimmunized recipients (B6 (Hi)). In generation B6 73.6 cent 40-chromosome cells were found in generation B6 (Hi) 36.8 per cent only. Furthermore in the triploid region generation B6 displayed a narrow range of variation (61-64) whereas the tumours of the hyperimmunized hosts showed a wide distribution (56-64). From generation B6 (Hi) transplantation was carried out to untreated hosts. A generation denominated B7 (HiU) showed in comparison with the preceding generation B6 (Hi) an increased number of 40-chromosome cells (56%)

Within the triploid region a narrower range of variation (59-64) was found. The parallel B7 generation on the other hand showed a reduced percentage of 40-chromosome cells (39.2%). Within the triploid region an almost similar range (58-64) as in B7 (Hi) was recorded. Finally transfer generations B8 and B8 (HiU) had a rather similar chromosome pattern.

Mean outgrowth period of the tumours per transfer generation (Figs 3-4) varied to some degree between the series. Of interest was a rather distinct variation between the B and the b series and a prolonged outgrowth period when using hyperimmunized mice. The B series was characterized by faster growing tumours with an outgrowth period

per generation ranging from 13.8 to 18.6 days while the b series displayed slower growing tumours with a mean value per generation ranging from 16.4 to 54.8 days. In generation b5 (Hi) a mean outgrowth period of 44 days was recorded in the parallel generation b5.22 days. Also when transplanting from the B series to hyperimmunized hosts B6 (Hi) a prolonged outgrowth period was observed in comparison with the untreated generation B6. The mean value for generation B6 (Hi) was 23.2 days and for generation B6.13.8 days only. However when retransplanting from hyperimmunized to untreated hosts once again faster growing tumours appeared. The mean outgrowth period of generation B7 (HiU) and the subsequent generation B8 (HiU) agreed well with the parallel generations B7 and B8. The mean values for the B (HiU) generations were 14.8 and 18.0 days respectively and for the Π generations 14.0 and 14.2 days.

Metacentric chromosomes. The percentage of cells with one or more metacentrics per transfer generation is recorded in Fig. 2. It should be noticed that besides generation B2 which showed 37 per cent cells with metacentrics high values were also recorded for transfer generations b3 (31.2%), b7 (36.2%) and b8 (40%).

Histologic classification. The primary tumour Π was classified as an osteosarcoma of predominantly mixed type containing cartilage components of varying degree of differentiation plus tumour bone and in the peripheral regions mainly cells of anaplastic type. Samples obtained from tumours of the B and b-generations had continuously more anaplastic cells but with the difference that the b tumours also displayed a focal osteoid formation. Because of a failure in preparation no analysis was performed on the tumours of the hyperimmunized generation B6 (Hi). In tumours of generation b5 (Hi) a moderate bone formation was found indicating a higher level of cellular differentiation. Along the bone trabeculae the cells resembled osteoblasts and in other areas were still more anaplastic.

Conclusions

A primary aim of the present investigation was to test to what extent the selection of tumour used for transplantation may affect the chromosome pattern in succeeding transfer generations but also to analyse whether tumour transplanted to hosts with a changed immunologic response would display a dif-



Fig. 5. A 48-chromosome cell from a tumour of transfer generation b5 (Hi).



Fig. 6. A 67-chromosome cell from a tumour of transfer generation B5.

ferent chromosome pattern in comparison with a tumour transplanted to untreated recipients. Another intention was to examine whether variations in outgrowth period could be observed when transfer series were established from a slow growing a fast growing tumour and when transplanting to hyper- and nonhyperimmunized recipients. The ages of the tumours used for transplantation were, except for transfer generation B2, selected so as to

representative as possible for the generations in order to avoid factors that may interfere with the evaluation of the results. Furthermore, the size of peripheral tumour pieces used for transplantation was standardized to the utmost possible extent. The B and b series displayed a different chromosomal progression (Table 1) and primarily in the triploid region. The B series was thus characterized by a predominating number of 60 to 70 chromosome cells, while the b series showed continuously lower chromosome numbers and with a mean at 54 to 57. Furthermore, the tumours of the B series often displayed a higher frequency of 40-chromosome cells. However, even if these results indicate differences in chromosomal progression, these should not be considered as a typical variation between slow and fast growing tumour series. It should be realized that coincidental changes may have caused this variation, especially with regard to often considerable differences between tumours of one and the same transfer generation and also between generations of a series. This circumstance may also explain why no direct effect was observed when transplanting tumours to hyperimmunized recipients, even if these tumours for instance displayed a smaller number of 40-chromosome cells than the tumours of the untreated hosts. Concerning the mean outgrowth period of the tumours per series, it was found that the mean value for the B series was 29 days and for the b series 29.0 days. However, in the latter series faster growing tumours appeared in the late generations. When transplanting to hyperimmunized recipients a prolonged outgrowth period was observed in comparison with the untreated parallel generations, but it was also noticed when retransplanting to untreated recipients that faster growing tumours were obtained. Thus, it can be ruled out that there may be a certain variation caused by difference in the immunologic response, but that this variation is not quite uniform as similarities were found between untreated and some hyperimmunized recipients. Nevertheless, the present results should indicate the need for some caution when evaluating at least transplanted radiation induced tumours.

SUMMARY

Highly inbred CBA mice were used. The registration of chromosome abnormalities was limited to numerical deviations and the occurrence of metacentric chromosomes. By separate serial transplantation from a ^{90}Sr induced osteosarcoma two parallel transfer series (B and b) were established. From these series transplantation was also performed to hyperimmunized hosts II (Hi) and b (Hi). Besides differences in mean outgrowth period between B and b generations, a variation in chromosome pattern was observed. However, this variation should not be evaluated as a typical chromosomal progression of fast and slow growing tumour series.

ACKNOWLEDGEMENTS

The author wishes to thank Mr Arne Berg and Mrs Karin Fredell for technical assistance and Mrs Siw Siljerud for technical assistance and excellent analysis at the microscope.

REFERENCES

- BERGMAN H. and NILSSON A.: Chromosome counts of ^{90}Sr induced osteosarcomas in mice. I. Transplanted tumour series. *Acta radiol. Oncology* 19 (1980) 17.
- KATO R.: The chromosome of forty two primary Rous sarcomas of the Chinese hamster. *Hereditas* 59 (1968) 63.
- MARK J.: Rous sarcomas in mice. The chromosomal progression in primary tumours. *Europ. J. Cancer* 5 (1969) 307.
- MITelman F.: The chromosomes of fifty primary Rous rat sarcomas. *Hereditas* 69 (1971) 155.
- NILSSON A.: Histogenesis of ^{90}Sr induced osteosarcomas. *Acta vet. scand.* 3 (1962) 185.
- Early development of transplanted ^{90}Sr induced osteosarcoma buds. *Acta radiol. Ther. Phys. Biol.* 4 (1966) 7.
- Dose dependent carcinogenic effect of radiostrontium. In: *Proceedings of a symposium on radiation induced cancer*, organized by the International Atomic Energy Agency, p. 173. IAEA STJ/PUB/228, Vienna 1969.
- and RÖNNBACK C.: Influence of oestrogenic hormones on carcinogenesis and toxicity of radiostrontium. *Acta radiol. Ther. Phys. Biol.* 12 (1973) 209.
- and ULLBERG S.: Uptake and retention of strontium 90 induced osteosarcomas. II. *Acta radiol.* 58 (1962) 168.
- REVEŠZ L. and ERIKSSON K. H.: Antigenicity of radiostrontium induced osteosarcomas. *Radiat. Res.* 52 (1972) 395.

GRANULOSA AND THECA CELL TUMORS

Incidence and occurrence of second primary tumors

ELISABET BJÖRKHOLM and CLAES SILFVERSWARD

Material and Methods

Since 1958 Sweden has operated a nation wide Cancer Registry to which it is compulsory to report all malignant neoplasms and also tumors of certain types irrespective of whether they are classed as benign or malignant. Granulosa and theca-cell tumors belong to the last mentioned category (Cancer Incidence in Sweden 1959-1965).

In the Registry granulosa and theca-cell tumors and certain other types of ovarian neoplasms (mostly virilizing tumors and Brenner tumors) are assigned the same microscopic code. A list of women with this special code was obtained. The original microscopic reports (furnished by the different hospitals) on which the diagnoses were based are filed in the Registry. After analysing these files it was found that 687 cases should be classified as granulosa-cell tumors, 249 as theca-cell tumors and 72 cases belonged to neither of these categories. The series thus comprised 936 cases of granulosa and theca-cell tumors reported to the Swedish Cancer Registry between 1958 and 1972, of these 4 per cent were detected at autopsy. The list from the Cancer Registry provided information on the date of birth, the name of the patient, the date of diagnosis, the mode of diagnosis (operation or autopsy), the reporting hospital and the date of diagnosis of any other malignant disease (until December 1973). In the case of death the date and cause of death were

Granulosa and theca-cell tumors constitute the most common types of the ovarian gonadal stromal tumors. The malignant potential of granulosa-cell tumors is a matter of debate and figures given in the literature for recurrences or metastases vary from less than 10 to more than 33 per cent (MORRIS & CULLY 1958). They contain granulosa and theca cells in varying proportions (TEILUM 1971). Pure theca-cell tumors, thecomas, are generally benign (MORRIS & CHORLTON 1974). The ratio of granulosa to theca cell tumors varies in different series (MIDDLE 1952). In a large compilation found the ratio to be 4:1, others have reported 1:1. The incidence rates for granulosa cell tumors in different countries and races vary from 0.05 to 1.70 per 100,000 female population (KOLSTAD & BEECHAM 1975). This large range is due at least in some measure to differences in the reporting of these lesions; for example, some reports seem to include pure theca cell tumors, others not. Incidence rates for thecomas are difficult to ascertain. The occurrence of endometrial carcinoma in patients with feminizing tumors of which granulosa and theca cell tumors constitute the main part, has been well documented (INGRAM & NOVAK 1951; MANSELL & HERTIG 1955; GREENE 1957; GUSBERG & KARDON 1971).

The incidence of granulosa and theca-cell tumors in the total Swedish female population was analysed and the numbers of new primary malignant tumors reported in women with granulosa and theca-cell tumors compared with the expected numbers by using data from the Swedish Cancer Registry. The results are now reported.

given (until December 1976). A closing date for collecting the series was set to December 1973.

From the Registry list—now containing only granulosa and theca cell tumors—every tenth case was taken for a morphologic re-evaluation. The hospitals reporting the patients were asked for the histologic specimens (slides) on which their initial diagnoses were based. This material was received for 87 cases of the 93 requested. These 87 cases will be referred to as the 10 per cent sample. If carcinoma of the endometrium had also been reported in a patient, the endometrial biopsies were sent for as well. As the discrimination between granulosa and theca cell tumors in the Cancer Registry was objectionable, all the calculations except the estimations of incidence rates for the two tumor types were based on the total series, that is granulosa and theca cell tumors taken together.

Statistical methods. The average annual crude (all ages) incidence rate was computed from the average annual number of cases 1958 to 1972 and the mean female population of 1965 was used as the mean population for the period.

The age specific person years at risk for contracting a new malignant disease was calculated from date of diagnosis of the ovarian tumor to date of death or the closing date of the series. Age specific person years at risk for developing an endometrial carcinoma was computed in the same way, with the exception that for patients developing endometrial carcinoma the date of diagnosis of that disease was stop date. By applying the age specific incidence rates obtained from Cancer Incidence in Sweden 1965 (the median year of the series) for different malignant diseases to the number of person years at risk, the expected numbers of these tumor types were calculated (Cancer Incidence in Sweden 1965). The life time risk of developing an endometrial carcinoma was estimated by using information from life tables of Sweden for 1963 to 1967 and from Cancer Incidence in Sweden 1965.

The ratio of observed to expected numbers of malignant lesions was defined as the relative risk. 95 per cent confidence limits for the relative risk were given based on the Poisson distribution.

Age specific incidence rate was calculated for each 5 year group as the annual number of cases per 100 000 of the mean female population in that age group. An age standardized incidence rate was calculated for each year. The figures were adjusted for age to a standard population (the 1970 Census).

Table 1

Cases initially diagnosed as granulosa cell tumors and at review considered as other tumor types

Diagnosis on review	No of cases
Arrhenoblastoma	1
Serous cystadenocarcinoma	1
Insulinoma	1
Carcinoid	1
Mesothelioma	1

The time trend was calculated over the period from 1959 onwards (omitting 1958, the first year the Cancer Registry operated) using sliding mean, the logarithms of the age standardized incidence each year.

Results

The 87 reviewed cases, i.e. the 10 per cent sample, agreed well with the whole Cancer Registry series concerning mean ages at diagnosis, 57 and 58 years respectively, and the ratio granulosa to theca cell tumors based on the initial reports, 3.0:1 and 2.8:1 respectively. On the basis of the review, 28 per cent of the 87 cases belonged to either the granulosa or the theca-cell tumor group. At the review, only 59 per cent of the cases initially reported as granulosa-cell tumor were considered to belong to that group; 29 per cent were re-classified as theca cell tumors and 11 per cent were considered to be other types of tumors (Table 1). In one case diagnosis was not possible because of extensive necrosis. Ninety five per cent of cases with a reported diagnosis of theca cell tumor were after re-evaluation of the histologic specimens considered to belong to that group. Five per cent were re-classified as granulosa-cell tumors as they contained a certain amount of epithelial strands of the granulosa-cell tumor type. Applying these figures to the total series, 936 patients, 421 should be considered as granulosa cell tumors and 431 as theca cell tumors. Based on these last mentioned figures, the average annual crude incidence rate 1958 to 1972 of granulosa-cell tumours was 0.72 and of theca-cell tumors 0.74 per 100 000 female population; the 95 per cent confidence limits in both instances are 0.55 and 0.90.

The mean age at diagnosis for the total series was 58 years and the range 7 months to 89 years (Fig. 1). The age specific incidence rate for

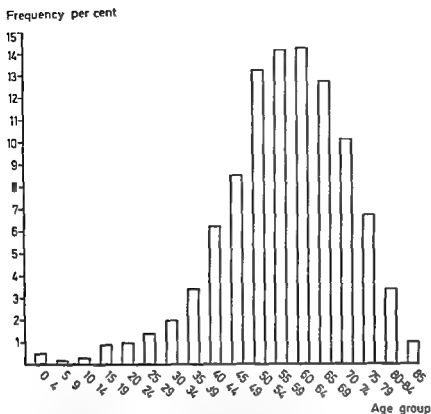


Fig 1 Age distribution of 936 women with granulosa and theca cell tumors reported to the Swedish Cancer Registry 1958 to 1972

group is given in Fig 2. An almost linear increase between 35-39 years and 65-69 years is found. The age standardized annual incidence rate and the trend appear in Fig 3. There seems to be an increased incidence until 1966 after which time it remained stationary. The correlation with time is positive ($r=0.4$) but not significant ($p=0.25$).

After the diagnosis of the granulosa or theca cell tumor the 936 women developed 55 new malignant diseases excluding other ovarian tumors (Table 2). The expected number is 42.8 (no statistically significant difference). In addition as many as 62 malignant tumors were found at the same time as the ovarian tumor, the majority (45) being endometrial carcinomas. The mean age at diagnosis of the ovarian tumor for the 45 women with concomitant endometrial carcinoma was 67 years. Five women with endometrial carcinomas reported after the diagnosis of the ovarian tumor had a mean age at diagnosis of the ovarian tumor of 43 years. The difference in mean age at diagnosis of the ovarian tumor for the two endometrial tumor groups is statistically significant ($p<0.001$). The mean interval between the ovarian diagnosis and the diagnosis of

the endometrial carcinoma was 11.9 years (range 4.6-13.5 years). The expected number of cases of endometrial carcinoma based on person years at risk was 2.6. In addition 11 women had a report of an endometrial carcinoma before the diagnosis of the ovarian tumor. If the life time risk of developing an endometrial carcinoma is calculated for the patients with granulosa or theca cell tumors (according to the microscopic review) the expected number would be 13.3. The total number of observed cases was 58 detected before, concomitant with and after the diagnosis of the ovarian tumor. This gives a relative risk of 4.4 (95 per cent confidence limits (3.3-5.6)).

Nine women with an endometrial carcinoma reported to the Cancer Registry were found in the 11 per cent sample. In 6 cases it was possible to confirm the diagnosis at review of the endometrial biopsies and one case was considered not to have an endometrial carcinoma. Two cases belonged to that group which was not considered to have this kind of ovarian tumor at the re-evaluation of the histologic specimens.

Fifteen mammary carcinomas were found after

No of cases per 100 000

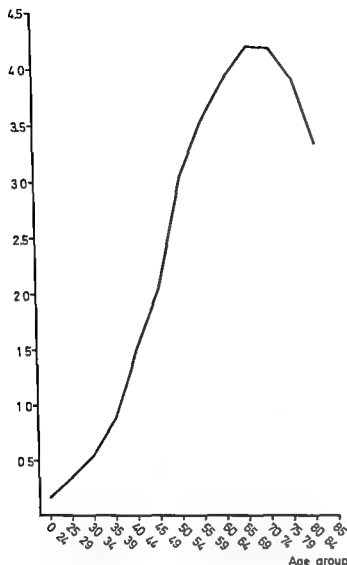


Fig 2 Age specific incidence rates for granulosa and theca-cyst tumours 1958 to 1977 per 100 000 of the mean female population

Table 2
New malignant tumors reported concurrently with
after diagnosis of ovarian neoplasm and the expected
numbers

Tumor site	Observed number		Expected (based years at risk time of diag- nosis of ovarian tumor)
	Reported after the ovarian tumor	Reported with the ovarian tumor	
Stomach	2	7	3.6
Small intestine	1	0	0.7
Colon	6	4	4.1
Rectum	3	1	1.9
Biliary ducts and liver	7	0	1.6
Pancreas	3	0	1.6
Breast	15	4	10.3
Uterine body	5	45	7.6
Uterine cervix	0	3	2.4
Kidney	1	0	1.5
Lower urinary tract	1	0	1.0
Melanoma of the skin	1	0	0.6
Skin	1	0	0.8
Brain	2	0	1.3
Thyroid gland	1	7	0.5
Other and unspecified locations	3	1	1.4
Malignant lymphoma	5	0	1.0
Multiple myeloma	1	0	0.6
Leukemia	2	0	1.0
All sites except ovary	55	62	47.8
All sites except ovary and uterine body	50	17	40.7

the diagnosis of the ovarian tumor compared with 10.3 expected. This difference is not statistically significant. However, if concomitant breast carcinoma is included, the difference is significant, the relative risk being 1.8, 95 per cent confidence limits (1.2-2.9).

The difference between observed and expected number of cases of carcinoma of the colon after diagnosis of the ovarian tumor was not statistically significant, but if concomitant colon carcinomas were included, the difference is statistically significant, the relative risk being 2.4, 95 per cent confidence limits (1.2-4.4).

The same conditions apply to the thyroid carcinomas, where no statistically significant difference

between observed and expected cases was found when concomitant cases were not taken into account. However, if tumors concomitant with ovarian tumor were included, the difference is statistically significant, the relative risk being 6.0, 95 per cent confidence limits (1.2-17.5).

The relative risk of contracting a malignant lymphoma was 5.0, 95 per cent confidence limits (1.6-11.7), thus the difference between observed and expected cases is statistically significant. Concomitant malignant lymphomas were observed.

No statistically significant difference between observed and expected numbers of leukemia was found.

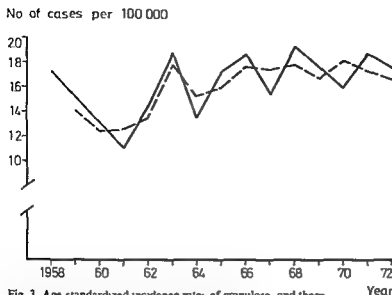


Fig 3 Age standardized incidence rates of granulosa and theca cell tumors 1958 to 1972 per 100 000 of the mean female population (solid line) and the trend (broken line)

Discussion

The calculations have been based on Registry figures. The reliability of such data may be discussed and as the Registry has many contributors the Swedish Cancer Registry is likely to contain some erroneously diagnosed cases (Cancer Incidence in Sweden 1973). The extent of possible errors in the diagnoses has not been analysed except in special research projects based on small numbers of selected types of tumors (MAŁEĆ *et al* 1977). No information on treatment was given despite of these elements of uncertainty it is probable that analyses of the present kind may shed light on the etiology of malignant tumors.

The review of the 10 per cent sample disclosed that only few errors of diagnosis were made when granulosa and theca-cell tumors were allotted to one and the same group. However the differentiation between these two tumor types was less reliable. Most errors affected the granulosa-cell tumor diagnosis. Based on the figures after reclassification the incidence rates of granulosa and theca cell tumors were found to be almost equal. In particular KOLSTAD & BEECHAM mentioned the remarkably high incidence of granulosa-cell tumors in Sweden as compared with other countries. This may to some extent be explained by the fact that in Sweden thecomas have been included in the calculations and to a minor part by errors made in the histologic classification. After making corrections

for the number of thecomas and incorrect diagnoses the incidence rate of granulosa-cell tumors in Sweden was still high compared with most other countries and approximately the same as that in Norway (KOLSTAD & BEECHAM). No change in incidence rates of granulosa and theca-cell tumors combined was found from 1966 onwards. The possible increase from 1959 to 1965 may depend on some uncertainty in the reporting of these tumors during the first years that the Cancer Registry operated. It is difficult to estimate the true incidence of these relatively benign neoplasms possibly at least some cases are not diagnosed. This may especially be true for the benign thecomas. Very few tumors in the present series were first diagnosed at autopsy. This fact may simply reflect a low frequency of post mortem examinations. The number of endometrial carcinomas in the 10 per cent sample was too small to draw any statistical inferences.

The mean age at diagnosis for this series is similar to that given previously (BURSLEM *et al* 1954, DIDDLE). It has been suggested that the maximum incidence of these feminizing ovarian tumors about the menopause should support a theory that pituitary gonadotrophins stimulate latent components of the ovary to tumor production (BURSLEM *et al*). In fact increased incidence rates were found with advancing age especially so from menopause at least when the very old age groups were not taken into account. This supports the theory mentioned

A nation wide series of granulosa and theca-cell tumors with a known back ground population and access to reports of new primary tumors made this analysis possible. A comparison with the expected number of the relevant tumors based on the age specific incidence rates for the Swedish female population has been possible. An analysis of new primary tumors may however be biased for a variety of reasons. One is the possibility of detecting one tumor as a consequence of the treatment of another. If as in the present material the ovarian tumor is comparatively benign and sometimes has a long duration before being diagnosed (BJÖRKHOLM & PETTERSSON 1980) the evaluation of concomitant tumors must be handled with special care. It is difficult to calculate the actual person years at risk and subsequently the expected number of tumors. Most patients having had one malignant disease will also be under careful medical supervision and if a new neoplastic lesion appears the possibility of detection must be greater than usual.

Granulosa and theca-cell tumors appear to have the capacity of producing endometrial change through prolonged unopposed or unmodified hormone stimulation by estrogen alterations or other endocrinopathies. The progress from normal endometrium to carcinoma under the influence of continuous endogenous or exogenous estrogen stimulation has been described (GUSBERG & KARLSON). The women in the present series ran a four times increased risk of developing an endometrial carcinoma as compared with the life time risk for the general population. This method of calculating gives a considerable under estimation of the true relative risk. However the life time risk was chosen for the comparison because of the complex problem of handling the large number of concomitant tumors and thus of reliably computing the expected numbers. On the other hand as most of the endometrial carcinomas were concomitant with the ovarian neoplasm treatment could not have had any major influence. The number of endometrial carcinomas observed after the diagnosis of the ovarian tumor seems to be high considering that an unknown but certainly not negligible number of women must have been hysterectomized at time of diagnosis of the ovarian tumor. Those who developed endometrial carcinoma after the ovarian diagnosis were significantly younger at time of diagnosis of their ovarian tumor than were the women with concomitant endometrial carcinoma. Here the usual concept that

continuous estrogen stimulation changes the endometrium into pre-cancerous and cancerous does not hold true. The probable excess estrogen production was discontinued by the removal of ovarian tumor and some 9 years later an endometrial carcinoma developed. While it is not known whether a recurrence of the ovarian tumor occurred in all women no deaths appeared among them from this cause. These patients may also have received radiation therapy. There may be a common etiological factor perhaps genetical or hormonal that may influence the development of both the ovarian and endometrial tumor.

An increased risk of new primary tumors has been found in patients with carcinoma of the lip (HORN & JAKOBSSON 1964) and ovarian carcinoma (REIMER et coll 1978) as first primary tumor. REIMER et coll reported an increased risk of second primary carcinomas of the uterine corpus or bladder, breast and hematopoietic system in follow up surveys of patients with ovarian carcinoma. In the present series carcinoma of the breast, colon and thyroid all occurred in excess or tumors concomitant with the ovarian neoplasm taken into account. The women were found to have a significantly higher risk of developing malignant lymphomas but no over risk for leukemias when in concordance with the mentioned report on ovarian carcinoma.

SUMMARY

An analysis of 936 women with granulosa and theca cell tumors reported to the Swedish Cancer Register between 1958 and 1972 showed crude annual incidence rates for these lesions of 0.72 and 0.74 respectively per 100,000 of the female population unchanged during the period. The age specific incidence rates both taken together increased almost linearly from 35 years. The women displayed an increased risk of developing endometrial carcinoma and malignant lymphoma possibly also breast, colon and thyroid carcinoma.

ACKNOWLEDGEMENTS

This investigation was supported by the Swedish Cancer Society and the King Gustaf V Jubilee Fund. The authors wish to thank Mr Bo Nilsson for valuable help with the statistical analyses.

Request for reprints: Dr Elisabet Björkholm, Department of Gynecologic Oncology, Radiumhemmet, S-141 86 Stockholm, Sweden.

REFERENCES

- ÖRKHOLM E and PETTERSSON F Granulosa cell and theca cell tumors To be published in Acta obstet gynaec scand
- RSLEM R W LANGLEY F A and WOODCOCK A S A clinicopathological study of oestrogenic ovarian tumours Cancer 7 (1954) 522
- DDLE A W Granulosa and theca cell ovarian tumours Prognosis Cancer 5 (1952) 215
- NHORN J and JAKOBSSON P Multiple primary malignant tumors Cancer 17 (1964) 1437
- REENE J W JR Feminizing mesenchymomas (granulosa-cell and theca-cell tumors) with associated endometrial carcinoma Amer J Obstet Gynec 74 (1957) 31
- USBERG S II and KARDON P Proliferative endometrial response to theca-granulosa cell tumors Amer J Obstet Gynec 111 (1971) 633
- GRAM J M JR and NOVAK E Endometrial carcinoma associated with feminizing ovarian tumors Amer J Obstet Gynec 61 (1951) 774
- OLSTAD P and BEECHAM J B Epidemiology of ovarian neoplasia In Diagnosis and treatment of ovarian neoplastic alterations p 56 Edited by H de Watterville Excerpta Medica Amsterdam 1975
- MALEC E EKLUND G and LAGERLÖF B Re appraisal of malignant melanoma diagnosis in the Swedish Cancer Registry Acta path microbiol scand (A) 85 (1977) 707
- MANSELL H and HERTIG A T Granulosa theca cell tumors and endometrial carcinoma Obstet Gynec 6 (1955) 385
- MORRIS J MCL and SCULLY R E Endocrine pathology of the ovary p 65 C V Mosby St Louis 1958
- National Board of Health and Welfare The Cancer Registry Cancer incidence in Sweden 1959-1965 p 34 Stockholm 1971
- The Cancer Registry Cancer incidence in Sweden 1965 Stockholm 1969
- The Cancer Registry Cancer incidence in Sweden 1973 Stockholm 1979
- NORRIS H J and CHORLTON I Functioning tumors of the ovary Clin Obstet Gynec 17 (1974) 189
- REINER R R HOOVER R FRAUMENI J F JR and YOUNG R C Second primary neoplasms following ovarian cancer J nat Cancer Inst 61 (1978) 1195
- TELLUM G Special tumors of ovary and testis and related extragonadal lesions Comparative pathology and histological identification p 173 Munksgaard Copenhagen 1971

PROLACTIN SECRETING PITUITARY ADENOMA

Observations in irradiated patients

A. DE SCHRYVER, D. VANDEKERCKHOVE and G. DEBRUYNE

Although isolated descriptions of galactorrhea and amenorrhea (or impotence) associated with the existence of a pituitary tumor were to be found in the literature more than half a century ago (BROWN 1925; AENEL 1928; KRESTIN 1932; FORBES et coll 1954) could define a syndrome characterized by galactorrhea, amenorrhea and low urinary gonadotrophins in 15 non acromegalic patients (8 of these with evidence of a pituitary tumor). It was subsequently known as the Forbes Albright syndrome. The tumors associated with this clinical entity were first thought to be chromophobe (FORBES et coll.) but subsequently, when more refined cytochemical methods were applied, were very often found to contain erythrosinophilic cells (HERLANT et coll 1965; PEAKE et coll 1969; LANDOLT 1973). There is now good evidence that (as had already been suggested by FORBES et coll.) the symptoms in these patients are to be explained on the basis of the increased prolactin secretion by the gland or the adenoma when present (FORSYTH et coll 1971; THORNER et coll 1974). In fact the incidence of adenomas in these patients is probably higher than would be expected on the basis of conventional lateral skull films. VEZINA & SUTTON (1974) found radiologic evidence of a pituitary tumor in 20 patients with serum prolactin increase, 14 having a sella of normal size. All tumors were confirmed at surgery.

The main reason why these patients seek medical advice is usually sterility (in men impotence) for

which the efficacy of 2-bromo- α -ergocryptine (bromocriptine Parlodel) medication has been well documented (THORNER et coll.; SHEARMAN & FRASER 1977). However, some cases do not respond satisfactorily to medical treatment (THORNER et coll.) and more important, a risk of accelerated tumor expansion exists during pregnancy (GENZELL 1975; SHEARMAN & FRASER; DE GENNES et coll 1978) with potentially grave ophthalmic complications as a consequence.

The short term (1.5 to 3 years) results in 6 patients treated with external irradiation are now reported and some of the problems relating to these data are discussed.

Material and Methods

The 6 women were referred by the Department of Gynecology of this hospital for a sella enlargement, high prolactin levels reaching 300 ng/ml or more, ophthalmologic symptoms or primary resistance to bromocriptine medication or any combination of these. Some of their relevant clinical data are summarized in Table 1. All had been treated previously with various drugs including clomiphene, human menopausal gonadotrophine, human chorionic gonadotrophine and bromocriptine for periods varying from 2 to 7 years. In Case 3 the symptoms developed after discontinuation of oral contracep-

Table 1
Pre irradiation data

Case No	Sex	Age at irradiation	Amenorrhea (years)	Galactorrhea	Sella abnormality	Visual fields	Previous treatment period (years) + 4 ovulation induction or bromocriptine
1	F	39	2	-	Erosion of floor	Bitemporal sup quadrantanopsia	7
2	F	27	4	+	Erosion of floor	Normal	4
3	F	23	2	+	Enlarged	Normal	2
4	F	26	-	+	Erosion of floor	Normal	2
5	F	27	4	+	Erosion of floor	Normal	2
6	F	6	11	+	Enlarged	Incipient sup quadrantanopsia	2

Table 2
Mean plasma LH, FSH, TSH, GH and cortisol levels during radiation therapy

	Normal range	Day 0 (pre irr.)	Day 4	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
LH	4-20 mIU/ml	9.6	10.5	8.3	6.1	6.5	7.5	7.0	3.8
FSH	6-15 mIU/ml	12.7	17.1	16.1	10.9	7.7	9.7	11.4	8.3
TSH	<5 μ U/ml	9.4	-	3.5	3.9	2.1	2.6	2.8	5.5
GH	1-5 ng/ml	0.1	-	0.25	0.3	2.8	0.7	1.8	0.1
Cortisol	80-280 ng/ml	251	-	139	150	129	210	153	115

tive treatment. All had an abnormal sella strongly suggestive of an intrasellar adenoma: uni or bilateral erosion of the sella floor to unambiguous enlargement of the sella. During this same period (Jan 1 1976 to Sept 30 1978) a further 23 women with hyperprolactinemia and amenorrhea or short luteal phase (14 among them with galactorrhea) were successfully treated medically and irradiation did not have to be considered. In only 2 of these was a sella floor asymmetry found.

Hormone assays. Prolactin was assayed by means of a double antibody radioimmunoassay technique using the NIH reference preparation as a standard. Serum prolactin levels of 5 to 25 ng/ml were considered normal. Other pituitary hormones were assayed in 3 patients (Cases 1, 2, 5) and included gonadotrophins (FSH), luteinizing (LH), thyroid stimulating (TSH) and growth (GH) hormones and cortisol. Their normal (fasting) ranges are given in Table 2. For FSH and LH the figures relate to the follicular phase of normally menstruating women.

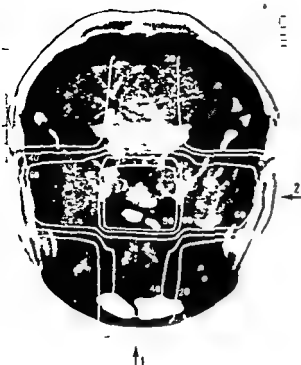
Radiation therapy. Patients were treated with 8 MV photons from a Philips SL75 linear accelerator

using a 3 field arrangement (Fig. 1). Radiation measured 4 cm \times 4 cm and their position centered on the pituitary fossa was carefully checked by means of routine simulator films. Daily partial doses of 1.8 Gy were administered up to a total of approximately 50 Gy in 51 weeks (cf. Table 3 for details). All 3 fields were treated each day.

Patients were controlled at frequent regular intervals but at least once every month and hormone assays were repeated as a rule to assess the effect of subsequent medication which was continued in all patients.

Results

Clinical data. In 3 of the 6 patients irradiated (Cases 1, 3, 5) it would seem as if radiation therapy followed by maintenance bromocriptine treatment had contributed to the reduction of serum prolactin to normal levels with the result that 2 of them eventually became pregnant 6 and 9 months respectively after completion of irradiation. Each of these two gave birth to a normal child in the third



1 Example of radiation dose distribution

mal menstrual cycle was re-established (with pregnancy no longer wanted). All 3 are still on maintenance bromocriptine medication (5 to 7.5 mg daily). In Case 1 the bitemporal superior quadrantanopia disappeared. The remaining 3 patients do seem to have benefitted from irradiation. In Case 6 the adenoma continued to grow with aggravation of her visual symptoms until one year after the irradiation course surgery had to be performed.

The lesion (a chromophobe adenoma) was removed trans sphenoidally during an uneventful operation. She is still under 5 mg bromocriptine and both her vision and prolactin levels have normalized as well as her menstrual cycle (2 years after surgery). The other two (Cases 2-4) are still amenorrheic one and 3½ years respectively after irradiation but no further treatment other than bromocriptine is being considered at the moment.

Radiation induced hormone level changes Before irradiation serum prolactin levels varied widely in the 6 cases and ranged from 70 to 790 ng/ml (median 255 ng/ml, Fig. 2) in contrast to the 23 patients who responded primarily to medication and were not irradiated (median 57 ng/ml). The 3 responders do not seem to have had any particularity in common: 2 of them had high initial prolactin levels (Cases 1 and 3) while Case 5 only had moderately elevated prolactinemia. Their response to the radiation therapy (Figs 2-3) was comparable to that of the others. Among the non responders only in Case 2 could it be said that a rather poor effect on prolactin levels was obtained (but no worse than that for Case 5 who responded). On the other hand in Case 6 the prolactin levels were more than halved during the radiation course although without clinical result. On the evidence of this small group of patients it would seem that prolactin variations during radiation therapy are probably of little help in predicting the result of the treatment. During irradiation prolactin levels showed after an initial rise in some a downward trend in every patient with end levels of 81, 68, 67, 43 and 31 per cent of

Table 3

Treatment data and clinical results

Case No	Irradiation data	Post irradiation bromocriptine maintenance treatment (mg)	Clinical result	Final prolactin levels (ng/ml)
1	55 Gy/42 d	7.5	Normalization of cycle, disappearance of quadrantanopia	15.2
2	50 Gy/37 d	17.5	No response	570
3	50 Gy/44 d	7.5	Pregnancy	121
4	50 Gy/42 d	7.5	No response	981
5	45 Gy/37 d	5.0	Pregnancy	23.4
6	50 Gy/47 d	5.0	Progressive growth of adenoma with deterioration of vision	<2 (post surgery)

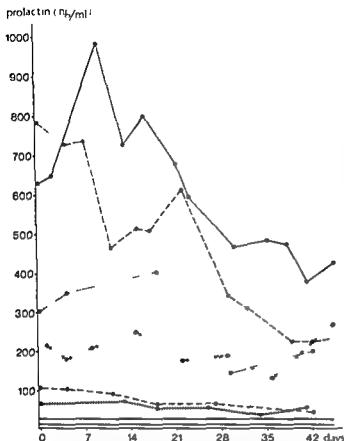


Fig 2 Serum prolactin variations in absolute figures during irradiation (45 Gy/37 days to 55 Gy/42 days) \square normal value Case 1 (---) Case 2 (+++) Case 3 (—) Case 4 () Case 5 () Case 6 (- -)

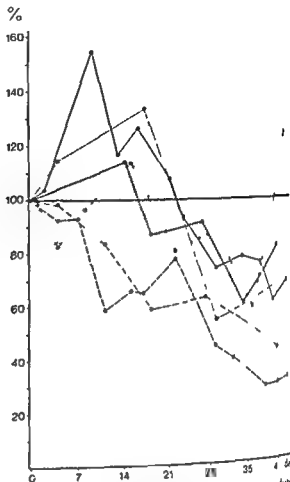


Fig 3 Serum prolactin variations (in per cent of pre irradiation value) during irradiation. Symbols as in Fig 2

pretreatment values. Case 2 ended up with a higher than pretreatment level (125%) and proved a non responder (even to bromocriptine). Among the others, no correlation was found between the degree of serum prolactin decrease and clinical response.

In 3 patients, other pituitary hormones were assessed during treatment for comparison with prolactin values (Table 2, Fig 4). It would seem that (with the exception of GH, which varied widely) FSH, LH, TSH and cortisol also had a similar downward trend as did prolactin, although by the end of the treatment they were still by and large well within normal range.

Discussion

Radiation therapy as a primary treatment for prolactin secreting pituitary adenoma has been used by KLEINBERG et al (1977). Their results did not seem promising. Of 8 patients treated, none became pregnant, none of 6 amenorrhoeic patients had a resumption of their menses although galactorrhea dis-

appeared in 3. But prolactin levels were reduced to an average of 26 per cent of pretreatment values. The authors concluded that they could find no clear superiority of either trans sphenoidal hypophysectomy or irradiation as a mode of treatment in the patients. SHEARMAN & FRASER treated 5 patients with (intrasellar) microadenomas with external radiation but found it too early to determine the result of this treatment.

The present data, admittedly on only 6 patients, show that prolactin secreting adenoma can respond to irradiation, probably in a substantial proportion of cases. They do not obviously permit any approximation on the definitive value of radiation therapy in the management of this condition, much less its comparison with primary surgery. They also raise a number of further questions which it may be of interest to comment upon in brief.

The first question is whether the radiation-induced prolactin decrease is due to a direct effect on the adenoma or to an indirect hypothalamic

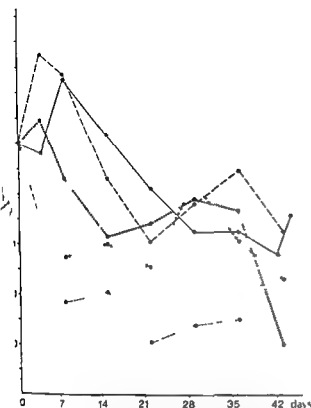


Fig. 4 Variations in serum FSH (---) LH (○) TSH (△) and prolactin (□) mean of 3 patients and serum prolactin (—) of 6 patients during radiation therapy in per cent of pre irradiation value

mediated effect. It has been suggested at least for some cases of pituitary tumor that the mechanism governing the prolactin hypersecretion might be related to interference with the pituitary stalk or hypothalamus (FRANTZ et coll 1972 FRANKS et coll 1977) and to the resulting loss of its prolactin inhibitory action. If for the sake of argument it is assumed that such a mechanism were at all involved in its pathogenesis then it is of course conceivable that this particular mechanism might be affected by radiation therapy. The data presented here do not permit a definite conclusion one way or the other however in view of the relatively early repercussion of the irradiation course on serum prolactin detectable at dose levels not considered particularly high (20 to 30 Gy) it may well be asked whether this may not have been mediated through changes induced at the hypothalamic rather than at the adenoma level. Growth hormone levels in acromegalic patients can respond to irradiation but this seems to be a process that will take months to years (ROTH et coll 1970). Pituitary irradiation for Cushing's disease may induce a decrease of serum

ACTH but even this process will take several months (ROSS et coll 1979). LARKINS & MARTIN (1973) described a case of hypopituitarism after radiation therapy developing in a patient given 65 Gy for a carcinoma of the maxilla and in whom they felt the pituitary insufficiency might have been of hypothalamic origin. However and although very little is known about the sensitivity of the hypothalamic structures (most of which are probably comprised within the target volume whenever the pituitary is irradiated) it seems unlikely that a dose of 50 Gy could result in a functional boost i.e. an increase in prolactin secretion inhibition. The observed serum prolactin decrease seems most likely to be explained on the basis of a direct effect of the radiation on the pituitary cellular level.

The second question is whether the radiation induced decrease of prolactin levels in these patients is a specific phenomenon or whether the other pituitary hormones are also influenced as well as the normal glandular function. The integrity of the various other pituitary axes could be confirmed only through the use of special stimulation tests which were not performed. However from a purely clinical point of view the answer to these questions was simple at least in a short term perspective no symptoms suggestive of impairment of any of the other pituitary functions were ever noticed. The sensitivity of normal adult pituitary tissue and hormone producing mechanisms have been known for a long time to be quite low. A recent literature review (FUKS et coll 1976) was found to mention only 2 adult cases of radiation induced hypopituitarism which was diagnosed 7 and 8 years after irradiation (65 and 60 Gy respectively). The authors suggested the possibility of long term damage on the basis of cumulative post mitotic cell death in a slow renewal type of tissue. Apart from the extreme rarity of reports of clinically established cases an analysis of 29 patients surviving as adults for 10 to 30 years after radiation therapy for malignant nasopharyngeal tumours and in whom the dose to the pituitary was estimated as ranging from 17 to 68 Gy did not reveal any case of unequivocally impaired pituitary function at least with the tests then in use (DE SCHRYVER et coll 1973). Therefore the risk even long term for clinically relevant injury to the glandular tissue within the irradiated normal pituitary probably is quite small.

It must be noted that the serum levels of most other pituitary hormones tested (the exception being

GH) decreased. Since roughly similar curves were found for FSH, LH, TSH and cortisol, this can hardly be fortuitous and irradiation must therefore in all probability and to some extent interfere with the secretion of these various pituitary hormonal products as well. However, either these decreased levels are merely transient or, if not, the decrease is not sufficient to affect their peripheral targets.

The third question is whether the radiation induced prolactin decrease is a dose related phenomenon and the proportion of responders increased by increased dosage. Again the limited number of cases does not allow a definite answer. The patient who received the lowest dose (45 Gy in 37 days) responded, as did the one with the highest dose (55 Gy in 47 days). Whether a higher dose than 50 Gy in 42 days would have cured the 3 remaining patients is moreover somewhat irrelevant since the choice of an optimum dose is wholly dominated by the sensitivity of the adjacent neural structures in general and by that of the optic pathways in particular. The optimum dose in fact is the highest dose which will not induce injury to the optic nerve. For many years recommended dose levels have usually ranged between 45 and 55 Gy in 4.5 to 5.5 weeks. As more and more experience is accumulating it has become increasingly clear that a total target dose not surpassing 50 Gy in 28 daily fractions is unlikely ever to exceed nervous tissue tolerance and to induce neurologic (mainly ophthalmic) complications (ARISTIZABAL et al 1977). In order to keep radiation therapy of pituitary adenoma a safe procedure (which seems mandatory in view of the benign character of most of these lesions), due consideration must be given to the proper time dose parameters and allowance made for a number of treatment failures, the true proportion of which has yet to be determined.

SUMMARY

Six women with clinical, biochemical and radiologic evidence of a prolactin secreting pituitary adenoma were irradiated with 50 Gy in 5½ weeks. In 3 of the 6 patients irradiation preceding maintenance bromocriptine medication was followed by a normalization of serum prolactin levels which resulted in a normal pregnancy in 2 and reestablishment of the menstrual cycle in the third. In 5 of 6 patients serum prolactin was substantially reduced by the end of the treatment but without reaching normal levels, although no correlation was found between the degree of this decrease and the clinical result.

REFERENCES

- ARISTIZABAL S, CALDWELL W L and AVILA J: The relationship of time-dose fractionation factors to complications in the treatment of pituitary tumors by radiation. *Int J Radiat Oncol* 2 (1977) 667.
- BROWN W L: Discussion on the uses and abuses of endocrine therapy. *Brit med J* 2 (1925) 1051.
- DE SCHRYVER A, LJUNGGREN J-G and BLANDIN R: Pituitary function in long term survival after radiation therapy of nasopharyngeal tumours. *Acta radiol Ther Phys Biol* 12 (1973) 497.
- FORBES A P, HENNEMAN P H, GRISWOLD G C and ALBRIGHT F: Syndrome characterized by galactorrhea, amenorrhea and low urinary FSH compatible with acromegaly and normal lactation. *J clin Endocr* 14 (1954) 265.
- FORSYTH I A, BESSER G M, EDWARDS C R W, FRANKS S and MYRES P: Plasma prolactin activity in inappropriate lactation. *Brit med J* 3 (1971) 225.
- FRANKS S, NABARRO J D N and JACOBS H S: Prevalence and presentation of hyperprolactinaemia in patients with functionless pituitary tumours. *Lancet* (1977) 778.
- FRANTZ A G, KLEINBERG D L and NOEL G L: Studies on prolactin in man. *Rec Progr Horm Res* 18 (1978) 527.
- FUKS Z, GLATSTEIN E, MARSAG W, BAGSHAW M A and KAPLAN H S: Long term effects of external radiation on the pituitary and thyroid glands. *Cancer* 37 (1976) 1152.
- GENZELL C A: Induction of ovulation in infertile women with pituitary tumors. *Amer J Obstet Gynecol* 1 (1975) 311.
- DEGENNES J L, TURPIN G, HESHMATI M, CESSER E and LAGOUY M: Adénomes à prolactine révisés thérapeutiques. 18 Observations. *Nouv Presse Méd* (1978) 1713.
- HAENEL H: Ein Fall von dauernder Milcheekretion bei Manne. *Munch med Wschr* 75 (1978) 761.
- HERLANT M, LAINE E, FOSSATI P and LINGUETTE M: Syndrome aménorrhée galactorrhée par adénome hypophysaire à cellules à prolactine. *Ann Endocr (Paris)* 26 (1965) 65.
- KLEINBERG D L, NOEL G L and FRANTZ A G: Galactorrhea: a study of 235 cases including 48 cases of pituitary tumors. *New Engl J Med* 296 (1977) 699.
- KRESTIN D: Spontaneous lactation associated with enlargement of pituitary: with a report of 2 cases. *Lancet* 1 (1932) 928.
- LANDOLT A M: Ultrastructure of human sella tumors. *Acta neurochir* (1973) Suppl No 23 p 51.
- LARKINS R G and MARTIN F I R: Hypopituitarism after extracranial irradiation: evidence for hypothalamic origin. *Brit med J* 1 (1973) 157.
- PEAKE G T, MCKEEL W, JARETT L and DAUGHADAY W H: Ultrastructural histology and hormonal characterization of a prolactin secreting pituitary tumor. *J clin Endocr* 29 (1969) 1101.
- ROSS W M, EVERED D C, HUNTER P, BEVINS M and COOK D and HALL R: Treatment of Cushing's disease.

with adrenal blocking drugs and megavoltage therapy of the pituitary Clin Radiol 30 (1979) 149

TH J GORDEN P and BRACE K Efficacy of conventional pituitary irradiation in acromegaly New Engl J Med 282 (1970) 1385

ARMAN E P and FRASER I S Impact of new diagnostic methods on the differential diagnosis and treatment of secondary amenorrhea Lancet I (1977) 1195

THORNER M O McNEILLY A S HAGAN C and BASSER G M Long term treatment of galactorrhoea and hypogonadism with bromocriptine Brit med J 2 (1974) 419

VEZINA J L and SUTTON T J Prolactin secreting pituitary microadenomas Amer J Roentgenol 120 (1974) 46

PRIMARY MUCOSAL MALIGNANT MELANOMA

Appraisal of role of radiation therapy

YUNG II SON

Primary malignant melanoma rarely arises in the oropharyngeal, gastrointestinal or urogenital mucosa and even more rarely in the central nervous system. The prognosis of malignant melanoma of the skin is largely influenced by the histogenetic features: thickness, size of the lesion, degree of lymphatic infiltration and lastly type of surgery or adjuvant immunotherapy (GOLDSMITH et coll 1970, SHAH & GOLDSMITH 1970, BLUMING et coll 1972, RIPPEY 1973, HANSEN & MCCARTEN 1974, FORTON et coll 1974, COHEN et coll 1977, HOLMES et coll 1977, EL DOMEIRI et coll 1978).

Meanwhile malignant melanoma of the mucous membrane is generally regarded highly lethal irrespective of therapeutic modality due to its hitherto controversial histogenesis, potential ulcercentricity as well as capricious metastasizing capability and high local recurrence rate (STOUT LATTES 1957, DE LA PAVA et coll 1963, RAVEN DAWSON 1964, DESAI & CAVAUGH 1966, TEARNS et coll 1966, COLLANTES et coll 1967, ACK & OROPEZA 1967, PICCONE et coll 1970, LAUFE & BERNSTEIN 1971, DAW 1972, FRABLE et coll 1972, DEUTSCH et coll 1974). Although it is far from convincing, the therapeutic norm tends to favor radical surgery throughout the literature (MINO et coll 1948, DAS GUPTA & D'URSO 1964, DESAI & CAVAUGH, NORRIS & TAYLOR 1966, TEARNS et coll, COLLANTES et coll, PACK & OROPEZA, YACKEL et coll 1970, BINGHAM et coll 1971, LAUFE & BERNSTEIN, MORROW & RUTLEDGE 1972, DEUTSCH et coll, MUSER & LINDNER 1974).

Incidence of primary malignant melanoma of the female genitalia is approximately 0.1 per 100,000 female population, 3 per 1,000 skin melanomas or less than 3 per cent of all malignancies of the vagina (DAS GUPTA & D'URSO, DEUTSCH et coll, RAGNI & TOBON 1974). To date, about 100 cases of vaginal malignant melanoma have appeared in English literature (MINO et coll, DAS GUPTA & D'URSO, NORRIS & TAYLOR, LAUFE & BERNSTEIN, DAW, DEUTSCH et coll).

DAS GUPTA & D'URSO reported 3 cases to the existing 30 cases and observed a 5 year survival of 9 per cent (or 3 of 33). They advocated complete vaginectomy, vulvectomy and groin dissection for a mid third lateral vaginal lesion. Less emphatically did they advocate pelvic exenteration for a superior third vaginal lesion.

MULLANEY (1961), accepting only 15 of the 24 reported cases in the literature, added one case of her own. After reviewing 13 cases, NORRIS & TAYLOR reported an 8 per cent 5 year survival by simple to wide excision with pelvic node dissection. COLLANTES et coll, after adding 3 new cases, concluded that local excision invariably failed both at primary and distant sites in a short time. DESAI & CAVAUGH were dubious about extended radical surgery because of unpredictability in the behavior of the disease.

DEUTSCH et coll collected 74 cases from the literature and added 5 cases of their own. Conced-

Submitted for publication 6 August 1979

ing to a high incidence of local and pelvic recurrence after radical surgery they mildly advocated radical surgery for a better chance of local control over simple excision alone. The 3 cases subjected to radical surgery died in 1½ to 4 years while the 2 cases treated by local excision and radiation died within 9 months.

According to FENN & ABELL (1973) and RAGNI & TOBON various surgical modalities ranging from simple excision to radical surgery equally gave rise to a 9 per cent 5 year survival. Reiterating therapeutic skepticism toward radical surgery LAUFE & BERNSTEIN observed death in most reported cases to ensue in 6 to 12 months. In 9 autopsy cases death was caused by either extensive pelvic recurrence or distant metastases.

Incidence of primary malignant melanoma of the gastrointestinal tract excepting the anorectal canal is considered non-existent but it is in fact due to the inability of diagnosis. Metastatic gastrointestinal melanoma is no longer a medical curiosity (DAS GUPTA & BRASFIELD 1964, NATHANSON et coll 1967, WILLBANKS & FOGELMAN 1970, MENUCK & AMBERG 1975, FRASER MOODIE et coll 1976). In their report of 7 cases FRASER MOODIE et coll estimated small intestinal metastases to be far more common than realized. Reporting 18 cases WILLBANKS & FOGELMAN quoted the slightly less than one per cent incidence of metastases (10 of over 1000 skin melanomas) from the series of DAS GUPTA & BRASFIELD.

There appear to be less than 200 cases of primary rectal malignant melanoma in the literature since the first description in 1857. STEARNS et coll reported 30 and PACK & OROPEZA reported 20 cases citing a 0.8 per cent incidence of all anorecto-colonic malignancies. HUSA & HOCKENSTEDT (1974) reviewed 4206 cases of anorectal tumor and found a 0.33 per cent incidence of primary melanoma. They attributed the uniformly poor prognosis to the stage of disease. The type of surgery appeared to exert little or no influence on the prognosis.

Primary malignant melanoma of the esophagus is even a rarer entity. To date there are less than 40 confirmed cases in the western literature since the first case report in 1895 (RAVEN & DAWSON BASQUE et coll 1970, PICCONE et coll FRABLE et coll MUSER & LINDNER KURZBAN et coll 1976, MÅNSSON & BERGE 1977). Of greater rarity is metastatic melanoma of the esophagus (WOOD & WOOD 1975). BUTLER et coll (1975) compiled 3

cases from the literature and they reported the fourth.

Concurring with DAWSON (1925) and WILLIS (1948) FOWLER & SUTHERLAND (1952) postulated that esophageal melanoma might represent one of the multicentric foci originating in the entire epithelial basal layers rendering permanent cure unobtainable. Histogenesis of esophageal melanoma was seriously questioned by STOUT & LATTES. This was not readily accepted as an entity until the 1960s when melanin laden cells were identified in the basal layers of the esophageal mucosa (DE LUCA & PAVA et coll RAVEN & DAWSON PICCONE et coll).

Experience with esophageal melanoma has been starkly grim throughout the literature. Half of the cases died of disease within 6 months with an average survival period of 7.4 months (BIRCHER et coll). PICCONE et coll reported the longest recorded survival to be 15 months and the shortest 7 months.

The value of radiation therapy for mucosal malignant melanomas other than oculo-conjunctival presentation is difficult to assess but conventional radiation therapy with standard fractionation scheme is considered to be inferior to radical surgery. Incurability of such tumor by radiation therapy was stressed by COLLANTES et coll. In reviewing 37 cases from the literature in their summary 4 of the 9 cases of vaginal melanoma treated with supervoltage irradiation and radio-plaque died of wide spread metastases within a year.

Several case reports contain poor experience with immunochemotherapy for mucosal melanoma in contrast to the encouraging outlook for skin melanoma (BLUMING et coll FRABLE et coll MORTON et coll ALEXANDER & CONE 1977). The role of immunochemotherapy for mucosal melanoma cannot be established with certainty at present time.

In support of the widely held view of the futility of radiation therapy for malignant melanoma HABERMAZ & FISCHER (1976) demonstrated a striking correlation between the degree of response and the size of individual radiation fraction. According to their observation in none of 11 lesions treated with less than 5 Gy (500 rad) per fraction even partial response occurred whereas in 18 of the lesions given 6 to 23 Gy per fraction significant better response was obtained. This observation led to the conclusion that such a large size fraction is required to overcome a large shoulder. They

ted this threshold fraction size to be in the neighborhood of 6 Gy. They further concluded that there might be little or no correlation between the response probability and total radiation dose.

The retrospective survey of the U.K. and Bourne data on malignant melanoma by HORN *et al.* (1978) was also in agreement with their view supporting the efficacy of a relatively few large fractions over many small fractions in local control of melanoma. Good tumor response was observed in 10 to 58 per cent with the fraction size of 4 to 8 Gy ($p < 0.01$).

In the present report is described one case each of primary malignant melanoma of the vagina and esophagus treated by weekly or twice weekly large fraction irradiation supplemented by protracted high dose brachytherapy for long term local control.

Case reports

Case 1 A 58-year-old female presented pigmented nodular lesions in the vagina. She was otherwise asymptomatic except for vaginal discharge. She underwent abdominal hysterectomy for benign uterine fibroid 13 years previously. Examination revealed a bluish purplish raised nodule of 1.5 cm diameter and 2 pin head sized flat lesions in the mid third of the anterior vaginal mucosa. No pelvic or groin nodes were appreciated. Examination of the entire body surface skin revealed no pigmented lesions. Excisional biopsy showed malignant melanoma of deeply invasive type. Nothing abnormal was found on chest films, bipedal lymphangiography, bone and liver spleen scintigraphy nor at urethro-cystoscopy, rectosigmoidoscopy or CT of the pelvis and abdomen.

Because of the tumor location and depth of invasion, hysterectomy, pelvic and groin node dissection were considered inevitable. On the other hand, the chance of complete freedom from recurrence seemed small according to the literature. Under these circumstances it did not seem illogical to attempt a maximum local control by irradiation.

Eight weekly doses of 6 Gy via 2 wedge lateral and a single anterior pelvic ports with a 6 MeV linear accelerator were given. The lesions regressed considerably in the ensuing 2 months.

Three months later she underwent vaginal radium aqueous application which imparted 40 Gy to the mucosal surface. In the following 6 weeks the lesion further regressed to measure 0.3 cm with faint colored pin head sized lesions.

About 5 months after the beginning of treatment five ^{125}I seeds (0.64 mCi) were implanted to the base of the large nodule and one seed each to the pin head sized lesions. A small composite isodose pocket of 300 Gy over the total decay time period was confirmed by the computer dosimetry.

At this writing (10 months after the implantation) she remains free of local recurrence, regional or distant metastases.

Case 2 A 63-year-old male was admitted with a 6 week history of substernal discomfort, left shoulder pain and weight loss. A tumor was found in the middle of the esophagus below the level of the carina. At operation it was found that the mediastinum, the liver and celiac axis were free of metastases. The tumor was removed and esophago-gastric anastomosis was carried out. The surgical specimen showed a 8.5 cm long variegated mass with a discrete 5.0 cm nodular mass in it. Microscopically the main mass consisted of leiomyoma but the nodular mass represented malignant melanoma. The melanomatous lesion was deeply invasive into the muscularis and the proximal margin of resection was transgressed by the tumor. Scrutiny of the body surface skin showed no melanomatous lesion.

He received 35 Gy to the esophageal surface mucosa by 6 twice weekly applications of a radium bouganage. Following the second bouganage a 2.5 cm soft non tender node developed in the right supraclavicular region. Excision of the node revealed metastatic malignant melanoma. Six weekly doses of 5 Gy were given to the lower neck and upper mediastinum.

He was controlled 2 years after beginning of treatment. Scintigraphy of bone, liver and spleen was then normal as well as chest film. No local recurrence was found at radiography of the esophagus. Clinically he remained in good health.

Discussion

Primary malignant melanoma of the esophagus or vagina is extremely rare, which is the cause that no therapeutic alternatives other than radical surgery have been exhaustively attested.

It is doubtful that radical exenterative surgery can completely control a locally advanced mucosal melanoma. Even if radical surgery can provide a better chance of local control, the prospect of cure cannot be substantially improved because of the multicentric histogenesis and capricious metastasizing behavior.

While the value of immunochemotherapy is yet to be confirmed, an appraisal of large fraction irradiation seems to be appropriate.

One case each of primary malignant melanoma of the esophagus and of the vagina are reported. Both cases received non-conventional weekly or twice weekly large fraction/high dose protraction radiation therapy following excisional biopsy or wide excision alone of the primary or regional metastasis.

Although long term observation is lacking, a large fraction of 5 to 6 Gy can be repeated with impunity 6 to 8 times on a weekly or twice weekly basis. No

acute morbidity or complication has arisen in either case

Such intermittent large fraction therapy may prove to be an effective adjunctive means in controlling a local mucosal melanoma

SUMMARY

Primary malignant melanoma of the vagina and esophagus are reported and the literature on the subject is extensively reviewed. The role of large fraction irradiation is appraised. In both cases, no exenterative surgery was imposed but instead, intermittent large fraction irradiation with protracted high doses was elected for local control.

ACKNOWLEDGEMENTS

The ^{125}I seeds were supplied by 3 M Radiation Therapy Products, Sunnyvale, California 94086, U.S.A., which is gratefully acknowledged.

REFERENCES

- ALEXANDER R. M. and CONE L. A. Malignant melanoma of the rectal ampulla. Report of a case and review of the literature. *Dis Colon Rect* 20 (1977) 53.
- BASQUE G. J., BOLINE J. E. and HOLYOKE J. B. Malignant melanoma of the esophagus. First reported case in a child. *Amer J Clin Path* 53 (1970) 609.
- BERGDAHL L., BOQUIST L., LILJEQUIST B., THULIN C. A. and TOVI D. Primary malignant melanoma of the central nervous system. *Acta neurochir* 26 (1972) 139.
- BINGHAM D. L. C., CHADSEY L. C., RANCHAND S. and DORIS P. J. Primary melanocarcinoma of the esophagus. *Canad med Ass J* 105 (1971) 607.
- BLUMING A. Z., VOGEL C. L., ZIEGLER J. L., MODY N. and KAMYA G. Immunological effects of BCG in malignant melanoma. Two modes of administration compared. *Ann Intern Med* 76 (1972) 405.
- BUTLER M. L., VAN HERTUM R. L. and TEPLICK S. K. Metastatic malignant melanoma of the esophagus. A case report. *Gastroenterology* 69 (1975) 1334.
- CLIFFORD J. H., MCCLINTOCK H. G. and LUBCHENKO A. E. Primary spinal cord malignant melanoma. Case report. *J Neurosurg* 29 (1968) 410.
- COHEN M. H., KETCHAM A. S., FELIX E. L., LI S. H., TOMASZEWSKI M. M., COSTA J., ROBSON A. S., SIMON H. M. and ROSENBERG S. A. Prognostic factors in patients undergoing lymphadenectomy for malignant melanoma. *Ann Surg* 186 (1977) 635.
- COLLANTES T. M., PRATT J. H. and DOCKERTY M. B. Primary melanoma of the vagina. *Obstet and Gynec* 29 (1967) 508.
- CONLEY J. J. Melanoma of the mucous membranes of the head and neck. *Arch Otolaryng* 99 (1974) 315.
- DAS GUPTA T. K. and BRASFIELD R. D. Metastatic melanoma of the gastrointestinal tract. *Arch Surg* 98 (1964) 969.
- and D'URSO J. Melanoma of female genitalia. *Surgynec Obstet* 119 (1964) 1074.
- DAW E. Primary melanoma of the vagina. *Am J Obstet Gynec* 112 (1972) 307.
- DAWSON J. W. The melanomata: their morphology and histogenesis. *Edinb med J* 32 (1975) 901.
- DE LA PAVA S., NIGOGOSYAN G., PICKREN J. W. and CARRERA A. Melanosis of the esophagus. *Cancer* (1963) 48.
- DESAI S. and CAVAUGH D. Malignant melanoma of the vagina. *Cancer* 19 (1966) 632.
- DEUTSCH M., FRIED A. B., PARSONS J. A. and SARTAN G. Primary malignant melanoma of the vagina. *Oncology* 30 (1974) 509.
- EL DOMEIRI A. A., DAS GUPTA T. K., TRIPATHI S. B. T. Y. and CRISPEN R. Adjunct chemotherapy and immunotherapy in high risk patients with melanoma. *Surg Gynec Obstet* 116 (1973) 230.
- FRANK M. E. and ABELL M. R. Melanomas of the vagina. *Obstet and Gynec* 41 (1973) 909.
- FOWLER M. and SUTHERLAND H. D. Malignant melanoma of the oesophagus. *J Path Bact* 104 (1973) 473.
- FRABLE W. J., KAY S. and SCHATZKI P. Primary malignant melanoma of the esophagus. An electron microscopic study. *Amer J Clin Path* 58 (1977) 659.
- FRASER MOODIE A., HUGHES R. O. and SHOREY B. A. SNAPE L. Malignant melanoma metastases to the alimentary tract. *Gut* 17 (1976) 206.
- GOLDSWORTHY H. S., SHAH J. P. and KIM D. H. Prognostic significance of lymph node dissection in the treatment of malignant melanoma. *Cancer* 76 (1970) 606.
- HABERNALZ H. J. and FISCHER J. J. Radiation therapy of malignant melanoma. Experience with high individual treatment doses. *Cancer* 38 (1976) 2258.
- HANSEN M. G. and MCCARTEN A. B. Tumor thickness and lymphatic infiltration in malignant melanoma of the head and neck. *Amer J Surg* 178 (1974) 557.
- HOLMES E. C., MOSELEY H. S., MORTON D. L., CULWORTH W., ROBINSON D. and URIST M. M. A rational approach to the surgical management of melanoma. *J Surg* 186 (1977) 481.
- HORNSEY S. The relationship between total dose, number of fractions and fraction size in the response of malignant melanoma in patients. *Brit J Radiol* (1978) 905.
- HUSA S. and HOCKENSTEDT K. Anorectal malignant melanoma. A report of fourteen cases. *Acta Chir Scand* 140 (1974) 68.
- KURZBAN J. D., MARSHAK H. H. and MAALANSKY A. Primary malignant melanoma of the esophagus. *J Gastroenterol* 65 (1976) 464.
- LAUFE L. E. and BERNSTEIN E. D. Primary malignant melanoma of the vagina. *Obstet and Gynec* 37 (1971) 148.
- MANSSON T. and BERGE T. Primary malignant melanoma of the oesophagus. *Acta path microbiol scand* (1977) 395.

- UCK L B and AMBERG J K Metastatic disease involving the stomach *Amer J dig Dis* 20 (1975) 61
- JO R A MINO V H and LIVINGSTONE R G Primary melanoma of the vagina with a review of the literature *Amer J Obstet Gynec* 56 (1948) 325
- ROW C P and RUTLEDGE F N Melanoma of the vulva *Obstet and Gynec* 39 (1972) 745
- STON D L EILBER F R HOLMES E G HUNT S KETCHAM A S SILVERSTEIN M J and SPARKS J C BCG immunotherapy of malignant melanoma: summary of a seven year experience *Ann Surg* 180 (1974) 635
- LANEY J Primary melanoma of the vagina *J Path Bact* 81 (1961) 473
- SHER D R and LINDNER A E Primary melanoma of the esophagus *Amer J dig Dis* 19 (1974) 855
- HANSON L HALL T C and FARBER S Biological aspects of human malignant melanoma *Cancer* 20 (1967) 650
- IRIS H J and TAYLOR H B Melanoma of the vagina *Amer J clin Path* 46 (1966) 420
- K G T and OROPEZA R A comparative study of melanoma and epidermoid carcinoma of the anal canal: A review of 20 melanomas and 29 epidermoid carcinomas (1930 to 1965) *Dis Colon Rect* 10 (1967) 161
- COLE V A KLOPSTOCK R LEVEEN H H and SIKAJ Primary malignant melanoma of the esophagus associated with melanosis of the entire esophagus: First case report *J thorac Cardiovasc Surg* 59 (1970) 864
- RAGNI M V and TOBON H Primary malignant melanoma of the vagina and vulva *Obstet and Gynec* 43 (1974) 658
- RAVEN R W and DAWSON J Malignant melanoma of oesophagus *Brit J Surg* 51 (1964) 551
- RIPPEY J J A review of the classification of malignant melanoma *S Afr med J* 47 (1973) 528
- SHAH J P and GOLDSMITH H S Incontinuity versus discontinuous lymph node dissection for malignant melanoma *Cancer* 26 (1970) 610
- STEARNS N W JR GRODSKY L HARRISON E G JR QUAN S and ROB C M Malignant anal lesions *Dis Colon Rect* 9 (1966) 315
- STOUT A P and LATTES R Tumors of the esophagus *In Atlas of tumor pathology Sect V Fasc 20* (1957) AFIP Washington D C
- WILLBANKS O L and FOGELMAN M J Gastrointestinal melanomasarcoma *Amer J Surg* 120 (1970) 602
- WILLIS R A Pathology of tumours p 899 Butterworth London 1948
- WOOD C B and WOOD R A M Metastatic malignant melanoma of the esophagus *Amer J dig Dis* 20 (1975) 786
- YACKEL D B SYMONDS R E and KEMPERS R D Melanoma of the vulva *Obstet and Gynec* 35 (1970) 625

HODGKIN'S DISEASE CLINICAL STAGES I AND II

Results of radical irradiation with or without chemotherapy

B. HERNI, H. EGHBALI, M. DURAND, A. DE MASCAREL, D. MAREE,
G. HERNI-SIMON, P. RICHAUD, J. CHAUVERGNE and C. LAGARDE

The introduction of lymphography in the staging of Hodgkin's disease has individualized stages I and II with lymph node involvement on only one side of the diaphragm. In 1965 the Paris Symposium regulated radical radiation therapy for these stages thereafter patients were submitted to this type of therapy. The results show that about half of the patients were cured. The failures can be attributed to the fact that complete remission had not been induced or that patients had recurrent disease. These failures prompted the Stanford Group to perform exploratory laparotomy with splenectomy in order to obtain a more precise surgical staging and to extend the field of irradiation in certain patients (ADIN et coll 1971). Nevertheless, even with surgical staging the percentage of failures after extended irradiation remained important. Therefore it seemed useful to attempt a management associating radiation therapy and adjuvant chemotherapy (ROSENBERG & KAPLAN 1975, ROSENBERG et coll 1978). The drawback of this solution is that patients with an overall good prognosis are submitted to surgical staging and long heavy treatments which are not without certain complications. The authors are presently attempting to decrease the burden of this medical management which is not easy, particularly if laparotomy is to be avoided (BERNADOU et coll 1978, Editorial Lancet II 1978).

The failures of radical irradiation used alone have prompted also other groups such as EORTC (1972) and this institute to use adjuvant chemotherapy in

tially without surgical staging. Previously preliminary results have been reported (LAGARDE et coll 1975) and now the results of 10 years of follow up are reported.

Material and Methods

Between January 1965 and October 1976 190 patients with suggested Hodgkin's disease clinical stages I and II were treated at this institute. Histologic slides were reviewed in 1976 for all cases and the diagnosis of Hodgkin's disease was confirmed for 177 patients only.

Seven other patients were excluded from the present analysis because they had not been irradiated. They were treated before the Symposium of Paris held in February 1965. 4 (between 69 and 85 years) because their general condition was poor due to age or another disease, one refused treatment. Six of these 7 patients died, one is alive and disease free after secondary irradiation. Thus 170 patients who had received radical irradiation according to the same procedures during the considered period remain for analysis.

All these patients were seen at this institute at the time of diagnosis or soon after and none had been treated previously. All diagnoses were established by biopsy and Hodgkin's disease was classified ac-

Table I
Main prognostic criteria

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total	42	24	104
Male/female	21/21	13/11	71/33
Age			
<40	27	16	71
40-60	8	2	26
60	7	6	7
Histologic type			
I	5	5	30
II	20	13	50
III	15	3	23
IV	2	3	1
Clinical stage			
I	9	6	31
II	33	18	73
Mediastinal involvement	17	12	65
Infra-diaphragmatic disease	2	3	8
General symptoms (B)	5	8	26
Extranodal involvement (E)	1	3	11
Tumor diameter > 5 cm	7	10	55
Good prognosis	7	0	31

cording to international nomenclature (Lukes et coll 1966). All patients underwent clinical radiologic and biologic examinations according to the lines of the Rye Symposium (1966) with chest radiography tomography of the mediastinum bilateral lymphography from the foot and usual hematologic and biochemical blood tests. No surgical staging procedures were performed. diagnostic laparotomy was performed in 3 patients only. All cases were thus classified in a clinical staging stage I one area involved stage II two or more lymphatic areas involved on only one side of the diaphragm. The main prognostic criteria issued from these pretherapeutic examinations appear in Table I along with their distribution following the three treatment regimens. On the whole 2 groups of patients are distinguished according to the data issued from the EORTC HI trial (TUBIANA et coll 1979). (1) So-called patients with good prognosis fulfilling all the following criteria: less than 40 years old lymphoid predominance or nodular scleriosis at microscopy stages I or II with mediastinal involvement no systemic symptoms (A) no extra nodal (E) involvement (2) So called

patients with poor prognosis with one or more of the opposite criteria

Treatment regimens were as follows. Initially all patients were treated with radical irradiation (R). After having observed many early failures with this treatment (1968) it was decided to submit patients with poor prognosis to an initial chemotherapy followed by the same radical irradiation (C + R). Later on the treatment was reinforced as follows: patients with particularly poor prognosis (especially those with bulky tumors) were given several courses of chemotherapy before a radiation; secondly patients in complete remission received one further course of consolidation chemotherapy; lastly since this sandwich chemotherapy + irradiation + chemotherapy (C + R + C) was well tolerated and gave improved results in patients with poor prognosis it was decided to submit all patients to this combined therapy (Herni et coll 1977). All treatments were given in a controlled but not randomized manner. The details of the different types of treatment given in each group of patients appear in Table 2.

Table 2
Treatments

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total number of patients	42	24	104
Irradiation			
Localized	5	1	4
Mantle	6	8	9
Mantle + lumbo-aortic	8	12	82
Inverted Y	—	3	8
Splenic	0	0	0
Booster	1	1	1
Chemotherapy			
1 course before irradiation	0	24	91
—3 courses before irradiation	0	0	5
4-6 courses before irradiation	0	0	6
1 course after irradiation	0	0	100

Table 3
Results

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total number of patients	42	24	104
Initial complete remissions	34	22	100
Initial failures	8	—	4
Relapses	14	7	5
Irradiated area	1	3	2
Marginal extension	3	1	3
Lymph nodes	9	1	1
Visceral	1	3	2
Persistent complete remissions	0	13	95

A few patients had two types of relapses

Almost all patients received irradiation with ⁶⁰Co at the dose of 35 Gy to 40 Gy in 3.5 to 4 weeks with a weekly dose of 10 Gy (2 Gy per day × 5 days). 40 Gy were given in the involved regions, 35 Gy in the adjacent areas. Because of 2 cases of myelitis during the first months and years following the treatment after 1968 the spinal cord was protected with a 15 cm wide shield for the posterior field when the dose was higher than 20 Gy. It appears that this shield

significantly decreases the dose absorbed by the spinal cord but the dose to the mediastinal lymph nodes is only slightly decreased. However this decrease has not yet been exactly calculated. Lumbo-aortic regions and mantle fields were more frequently irradiated in the C + M + C regimen (Table 2). Except for 4 elderly patients and one pregnant woman mantle + lumbo-aortic regions were irradiated at the same time.

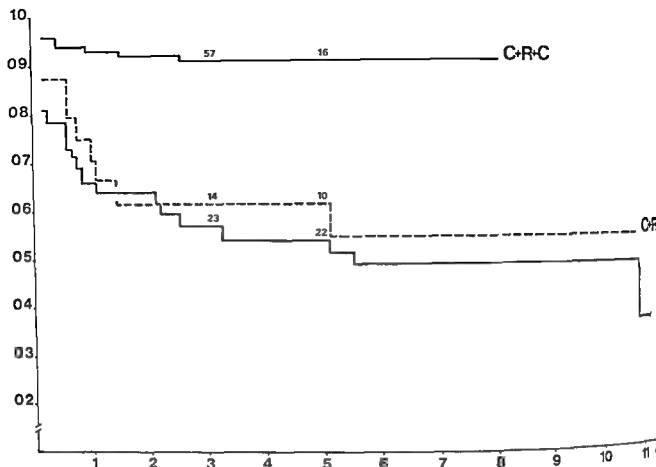


Fig 1 Actuarial recurrence free survival (a few patients in complete remission died of intercurrent disease) R = irradiation

alone C + R = chemotherapy + irradiation C + R + C = chemotherapy + irradiation + chemotherapy

Chemotherapy was usually an association of cyclophosphamide (200 mg every other day) + vinblastine (10 mg per week) + procarbazine (300 mg per day) + methylprednisolone given according to a previously published schedule (CHAUVERGNE et coll 1973) the treatment was discontinued as soon as the patient's leukocyte count dropped to $2 \times 10^9/l$ usually before the 21st day thus the treatment was usually shorter for the consolidation course than for the induction course. Most patients submitted to this schedule required hospitalization. Patients with bulky tumors who had received several courses of chemotherapy did not receive the treatment mentioned which is too poorly tolerated when repeated more than two times, they received the well known MOPP schedule (DE VITA et coll 1970).

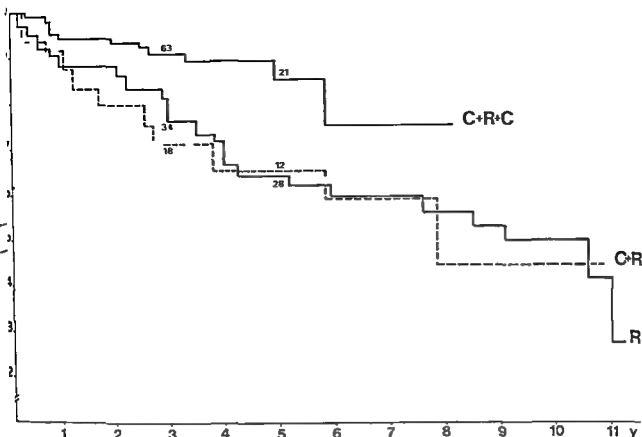
Radiation therapy was initiated in all patients immediately after the end of the initial 15 to 20 day course of chemotherapy. A rest period of one month was inserted between the end of irradiation and the beginning of the consolidation chemotherapy the average duration of this rest period was 33 days (for

5 patients chemotherapy could not be repeated two months later as the leukocyte count was not satisfactory after only a one month rest period).

No maintenance treatment was given after irradiation or consolidation chemotherapy administered as described. Thus the overall treatment was brief: one month when irradiation was given alone (R) 6 to 7 weeks when chemotherapy + irradiation were administered (C + R) about 3 months when patients given chemotherapy + irradiation + chemotherapy (C+R+C).

In case of relapse patients were re-treated according to the best currently available treatment.

Except for one patient who was lost to follow up all were followed up until their death or until the end of the study in the months of 1978. The follow up period is thus more than one year for all patients. All data were collected during May 1978. They were transcribed on a standard form for each patient. Survivals were calculated according to the actuarial method and significance according to the log rank test (P.E.T.).



2 Actuarial crude survival Symbols as in Fig 1

11 1977) Crude survival is considered from the date of diagnosis. Disease free survival is considered from the last day of radiation therapy.

Results

Complete remission was obtained in 34 of 42 patients given irradiation alone and in 122 of 128 patients given initial chemotherapy followed by irradiation. This significant difference ($p < 0.01$) appears in Fig 1 along with the actuarial curve of complete remission or recurrence free survival (all patients in complete remission died of intercurrent disease). This figure shows that patients given radiation alone have a plateau after 6 years (except for one patient who relapsed at 11 years). The disease free survival of so called patients with poor prognosis initially given chemotherapy followed by radiation is quite the same. The curve of patients treated with a sandwich schedule (C + R + C) is very different: no relapse occurred beyond 3 years and 57 patients have exceeded this period. The plateau is above 90 per cent (at 0.9087 exactly) if

can thus be expected that 90 per cent of these patients are cured.

The types of relapses are shown in Table 3. All types of relapses are reduced in patients given combined treatments; however relapses in distant lymph nodes are particularly frequent in patients treated with irradiation alone. The decrease in this latter type of relapse may be due to the fact that the lumbo-aortic area was irradiated more often in the combined regimen. If patients with good prognosis and poor prognosis are considered separately in the first group 2 failures of 7 R patients occurred and none of the 31 C + R + C patients. In the second group 21 failures of 35 R patients, 9 failures of 24 C + R patients, and 9 failures of 73 C + R + C patients occurred.

A few patients who did not respond or who relapsed obtained complete remission by another treatment and remained in complete remission with a follow up of 2 years or more: 4 of 22 failure R patients, 1 of 9 C + R patients and 3 of 9 C + R + C patients. Thus it appears that it is rare to cure a patient after a primary failure or a relapse.

Table 4
Course and survival

	Type of treatment		
	Irradiation alone	Chemotherapy + irradiation	Chemotherapy + irradiation + chemotherapy
Total number of patients	42	24	104
Total cumulative follow up (months)	3 139	1 364	4 587
Intercurrent diseases			
Herpes zoster	7	6	21
Other infections	1	1	9
Therapeutic complications	5	1*	6
Second malignancy	2	1	11
Hemolytic anemia	1	1	1
Miscellaneous	3	0	7
Normal pregnancies	5	0	4
Deaths			
from Hodgkin's disease	17	11	5
Therapeutic complication	1	1	1
Second malignancy	2	0	4
Other	2	2	1

* myelitis 3 fibrosis of brachial plexus 1 hypothyroidism

Femoral osteonecrosis

5 transitory bone marrow aplasia 1 intestinal complication 1 pneumothorax

Survivals appear in Fig. 2. These are uncorrected survivals including all causes of death (which are detailed in Table 4). These results are poorer than those for recurrence free survivals. About three fourths of the patients (28/40) with primary failures or relapses died; this proportion is slightly lower in patients who had been treated with a combined treatment (11/18) than in those who had been initially treated with irradiation alone (17/22); however this could be due to a shorter follow up period for patients given chemotherapy. It is striking that while Hodgkin's disease is the first cause of death in patients given irradiation alone (17/23) it accounts for only 5 of 12 deaths in patients who received sandwich treatment. Among other causes of death a second malignancy has a prominent place.

No immediate serious complication due to treatment was observed. The detailed hematologic tolerance of the sandwich regimen was presented elsewhere (EGHBAL et coll. 1978). Some severe delayed and eventually lethal complications were observed in the three groups of patients (Table 3). In 2 patients myelitis occurred after irradiation per-

formed without protection of the spinal cord (LEFRY et coll. 1971). In the sandwich regimen Späth developed a severe bone marrow aplasia at the end of the last course of chemotherapy but they recovered without complication (2 of them with antibiotics) within 10 days.

Table 4 shows other pathologic conditions observed in these patients. Herpes zoster was the most frequent infection; however it was not more frequent in patients given chemotherapy than in patients without chemotherapy (AUBERTIN et al. 1978). On the contrary other infections seemed to be more frequent in the third group of patients; this may be due to a longer period of follow-up. The only infection related to management of Hodgkin's disease was a pneumococcal septicemia supervening in a 15 year-old boy who had undergone laparotomy and splenectomy because of suggested relapse 4 years after initial treatment. Out of the therapeutic complications 4 were lethal (2 post irradiation myelitis, 1 bone marrow aplasia following the treatment of a relapse, and the septicemia mentioned). A second malignancy was observed 9 times in

Table 5
Nine secondary malignancies

Type of treatment	Type of malignancy	Delay between treatment and second malignancy	Localization in an irradiated region	Sex and age
Irradiation alone	Esophageal carcinoma	94 years	Yes	F 36
	Esophageal carcinoma	8 years	Yes	M 74
Chemotherapy + irradiation	Skin carcinoma	7½ years	No	F 77
Chemotherapy + irradiation + chemotherapy	Acute myeloblastic leukemia	5½ years	—	M 35
	Laryngeal carcinoma	2½ years	No	M 54
	Bronchial carcinoma	3 years	Yes	
	Osteogenic sarcoma	5 years	Yes	M 18
	Ovarian immunoblastosarcoma	8 months	No	F 19
	Bronchial small cell carcinoma	7 years	Yes	M 26

Patients with 5 carcinomas seem to be unrelated to treatment: one esophageal in an elderly alcoholic patient; one double (larynx + lung) in a heavy smoker; one skin carcinoma in an elderly patient with one immunoblastosarcoma supervening only 8 months after the initial diagnosis of Hodgkin's disease (Table 5). Such secondary non-Hodgkin's lymphomas were recently reported but always after an interval of over 5 years (KRIKORIAN et coll 1979). In other malignant tumors may result from the treatment given for Hodgkin's disease: they were more frequent in the third group of patients. Three malignant tumors may be related to the treatment among the 104 C + R + C patients or more accurately among the 95 patients apparently cured from Hodgkin's disease after C + R + C treatment: 2 of them occurred in an irradiated area (bronchial carcinoma and clavicle osteosarcoma).

Discussion

It is difficult to compare the results obtained with the three therapeutic regimens since they were not randomized. Moreover patients had been allocated to the second regimen (C + R) because of the presence of poor prognosis criteria. The first group of patients received irradiation alone and the results in this group are comparable to results obtained in other somewhat older series of patients who had been

staged only by radiologic and clinical procedures and irradiated in the same manner (EASSON 1966; KAPLAN 1966). It is more difficult to compare the results of the C + R + C regimen with other recent series because most of these series include patients after surgical staging. However the results obtained with this regimen seem valuable and should thus be considered when the best therapy in a similar type of patients is discussed. Similar results were obtained without laparotomy and extended radiation therapy but only for patients with clinical stage IA and IIA (GRIFFIN et coll 1977) or with a longer period of chemotherapy (ANDRIEU et coll 1979).

The C + R + C regimen is fairly well tolerated considering it is somewhat heavy. Hospitalization is usually required during chemotherapy but the overall treatment is quite brief. Large mantle and lumbo-aortic irradiation can be given at the same time immediately after the first course of chemotherapy without any major complication. The hematologic effects of this regimen have been detailed elsewhere (EGHBALI et coll). No deaths directly related to the initial treatment occurred. The only serious complication is second malignancy observed after association of chemotherapy and irradiation as noted in other series (ARSENEAU et coll 1977; BRODY et coll 1977). In comparison with other series the overall management is also lightened since neither surgical staging nor maintenance chemotherapy are needed. Moreover the spleens of these patients

were not surgically removed or irradiated and no unfavorable consequences were observed

Thus it seems difficult to improve the results. About 5 per cent (6/128) of the patients were initially resistant to treatment (both chemotherapy and radiation therapy) and were unable to obtain complete remission. Moreover 5 per cent of the patients (5/104) relapsed since relapses are varied it is difficult to propose any preventive therapeutic measure. Only 4 of 18 patients initially treated by C + R or C + R + C with initial or secondary failures had been treated subsequently more heavily and are now without evidence of disease and apparently cured. Therefore it must be pointed out that these rather good results are obtained with a heavy but brief treatment which does not require surgical staging, removal or irradiation of the spleen or long term chemotherapy.

However second malignancies appear with a low but threatening frequency. Some of them are certainly favored by the association of chemotherapy and irradiation. It is therefore necessary to try to alleviate the therapeutic regimen. It has been suggested that the radiation doses could be reduced after chemotherapy 15 to 20 Gy might be sufficient (PROSNITZ 1976). The suppression of chemotherapy would be particularly useful in young male patients because this treatment is often responsible for azoospermia (CHAPMAN et coll 1979). This endeavor is necessary at least in patients with good prognosis. In this latter group no failures occurred in 31 patients given the C + R + C treatment and very few failures in other patients more lightly treated. However even in this category of patients with good prognosis the percentage of relapses observed in the H1 trial after radical irradiation with or without vinblastine maintenance chemotherapy was slightly more than 25 per cent (TUBIANA et coll). There are reasons for reluctance in exposing patients to relapse since the possibilities of cure after relapse although not negligible are small (WELLER et coll 1976, DE VITA 1977). One way is to submit patients with good prognosis to exploratory laparotomy so that a more accurate prognostic evaluation could be obtained; this is currently being done by the Radiotherapy-Chemotherapy Group of the EORTC. Another way is rather to reduce the irradiated fields: such a randomized trial is now underway in the Pierre-et Marie Curie Group in France.

In summary the results show that it is possible to

give these patients a heavy but short treatment without surgical staging and splenectomy or spleen irradiation and without long term chemotherapy, and to obtain about 90 per cent of cure. However the treatment is still to be lightened mainly for patients with good prognosis in order to reduce the occurrence of complications mainly second malignancy.

SUMMARY

From 1965 to 1976 170 patients with Hodgkin's disease clinical stages I or II were treated with radical irradiation. A first group of 42 patients was treated with irradiation alone, a second group of 24 patients received one course of chemotherapy followed by irradiation and a third group of 104 patients was treated with a sandwich regimen including chemotherapy + irradiation + chemotherapy. Although the treatments were not randomized the comparison of the results shows a striking improvement in the group submitted to the C + R + C regimen: in this group the plateau of disease free survival was 90 per cent. The result was obtained after treatment for about 3 months without surgical staging or maintenance chemotherapy. However this treatment may favor some complications: mainly the development of a second malignancy. These risks may be decreased by reducing the treatment in patients with good prognosis.

The article was presented in part at the XVth Congress of the International Society of Hematology Paris 1978.

Request for reprints: Professor II Herni, Fondation Bergonie, 180 rue de Saint-Genès, F 33076 Bordeaux, Cedex France.

REFERENCES

- ANDRIEU J, M. BAYLE, WEISGERBER C, BOIRON V, BRIERE J, F. CLOT, P. DANA, M. JACQUILLAT, C. KATZ, M. and TEILLET F. Chemotherapy radiotherapy sequence in the management of Hodgkin's disease. Results of a clinical trial. *Europ J Cancer* 15 (1979) 153.
- ARSENEAU J, C. CANELIOS, G. P. JOHNSON, R. and DE VITA V. T. Risk of new cancers in patients with Hodgkin's disease. *Cancer* 40 (1977) 1917.
- AUBERTIN J, LACUT J, Y. HERNI, B. and DE KNOU, Opportunistic infections in cancer patients. Vol I. Masson, New York 1978.
- BERNADOU A, BLANC C, M. JAMES J, M. DIOC Z, TOUN R. et BILSKI PASQUIER G. Maladie de Hodgkin. Valeur des critères dits de pronostic et de l'association radio-chimiothérapique dans les stades lymphoïdes localisés (I et II). 94 malades laparotomisés. *Nouv Presse méd* 7 (1978) 807.
- BRODY R, S. SCHOTTENFELD D. and REID A. Multiple primary cancer risk after therapy for Hodgkin's disease. *Cancer* 40 (1977) 1917.
- CHAPMAN R, M. SUTCLIFFE S, B. MEES L, H. EMMETT

- C R W and MALPAS J S Cyclical combination chemotherapy and gonadal function: Retrospective study in males *Lancet* 1 (1979) 285
- AUVERGNE J HERNI B HERNI SIMON G DURAND M et LAGARDE C Chimiothérapie de la maladie de Hodgkin associant procarbazine, vinblastine, cyclophosphamide et méthylprednisolone. Analyse d'un série de 124 cures *Z. Krebsforsch.* 80 (1973) 179
- VITA V T Jr Hodgkin's disease: The salvage of radiation treatment failures *Int J Radiat Oncol Biol Phys* 2 (1977) 1035
- SERPICK A A and CARBONE P P Combination chemotherapy in the treatment of advanced Hodgkin's disease *Ann Intern Med* 73 (1970) 881
- SSON E C Long term results of radical radiotherapy in Hodgkin's disease *Cancer Res* 26 (1966) 1244
- Storral Staging laparotomy for Hodgkin's disease: Reassessment *Lancet* II (1978) 875
- HBALIH HERNI SIMON G DURAND M CHAUVERGNE J TOUCHARD J and HERNI B Hodgkin's disease treated by chemotherapy and large field irradiation: Hematologic effects *Acta radiol Oncology* 17 (1978) 289
- IRTC Radiotherapy/Chemotherapy Group A randomized study of irradiation and vinblastine in stages I and II of Hodgkin's disease: Preliminary results *Europ J Cancer* 8 (1972) 353
- IFFIN T GERDES A PARKER R TAYLOR E HAFFERMAN M TAYLOR W and TESH D Are pelvic irradiation and routine staging laparotomy necessary in clinically stages I_A and II_A Hodgkin's disease? *Cancer* 40 (1977) 7914
- ARY P CASTAINGS G HERNI B et TOUCHARD J La myélopathie progressive post radiothérapique tardive *J Neurol Sci* 14 (1971) 325
- ERNI B CHAUVERGNE J and LAGARDE C The strategy of treatment of Hodgkin's disease *In* Recent result in cancer research Vol 62 p 110 Edited by G Mathe Springer Berlin 1977
- UDIN M E GLATSTEIN E and DORFMAN R F Clinicopathological studies of 117 untreated patients subjected to laparotomy for the staging of Hodgkin's disease *Cancer* 27 (1971) 1277
- KAPLAN H S Long term results of palliative and radical radiotherapy of Hodgkin's disease *Cancer Res* 26 (1966) 1250
- KRIKORIAN J G BURKE J S ROSENBERG S A and KAPLAN H S Occurrence of non Hodgkin's lymphoma after therapy for Hodgkin's disease *New Engl J Med* 300 (1979) 452
- LAGARDE C CHAUVERGNE J DURAND M HERNI B HERNI SIMON G et TOUCHARD J Interet d'une chimiothérapie complémentaire de la radiothérapie dans les stades I et II de la maladie de Hodgkin *Bull Cancer* 62 (1975) 1
- LUKES R J CRAVER L F HALL T C RAPPAPORT H and RUBEN P Report of the nomenclature committee *Cancer Res* 26 (1966) 1311
- PETOR PIKE M C ARMITAGE P BRESLOW N E COX D M HOWARD S V MANTEL N MCPHERSON K PETO J and SMITH P G Design and analysis of randomized clinical trials requiring prolonged observation of each patient II Analysis and examples *Brit J Cancer* 35 (1977) 1
- PROSNITZ L R Radiation doses following intensive chemotherapy in the treatment of Hodgkin's disease *Int J Radiat Oncol Biol Phys* 1 (1976) 803
- ROSENBERG R A and KAPLAN H S The management of stages I and II of Hodgkin's disease with combined radiotherapy and chemotherapy *Cancer* 35 (1975) 55
- GLATSTEIN E J and PORTLOCK C S Combined modality therapy of Hodgkin's disease: A report on the Stanford trials *Cancer* 42 (1978) 991
- Symposium Obstacles in the control of Hodgkin's disease *Cancer Res* 26 (1966) 1046
- Symposium La radiothérapie de la maladie de Hodgkin *Nouv Rev franç Hemat* 6 (1966) 1
- TUBIANA M HENRY AMAR M HAYAT M BREUR L VAN DER WERE MESSING B and BURGESS M Long term results of the EORTC randomized study of irradiation and vinblastine in clinical stages I and II of Hodgkin's disease *Europ J Cancer* 15 (1979) 645
- WELLER S A GLATSTEIN E J KAPLAN H S and ROSENBERG S A Initial relapses in previously treated Hodgkin's disease I Results of second treatment *Cancer* 37 (1976) 2840

CHLORAMPHENICOL TOXICITY IN RADIATION DISEASE

M. POSPISIL, L. BENEŠ, L. TKADLEČEK, J. VACHA, V. VELČOVSKÝ,
J. NETIKOVA and S. VIKLICKÁ

In view of the role of bacteremia or bacterial sepsis in the pathogenesis of lethality following irradiation, antibiotics range among significant means of a complex therapy of the radiation disease. However, drugs which might damage the bone marrow and thus aggravate the radiation induced depression of the hemopoietic functions are contraindicated. These drugs may also include chloramphenicol (CAP) which is a potentially myelotoxic agent (UNIS 1969). It has been the aim of the experiments reported to confirm the possibility of a risk associated with CAP application in the radiation disease. Care has been taken to choose such experimental conditions that would correspond to high therapeutic doses of the antibiotic and its administration at a time when the protective abilities of the organism have declined (CRONKITE & BOND 1960). The experiments have been conducted on mice employed for analysing the CAP hematotoxicity (TRAVÉ et al. 1974).

Material and Methods

Male mice of the C₅₇Bl/10 strain were used. The animals were irradiated at the age of 12 to 13 weeks with an average body weight of 25 g. Standardized diet and tap water were given ad libitum. The mice were irradiated with a TUR apparatus operated at 180 kV, 5 mA, filtration 0.5 mm Cu and 0.5 mm Al, dose rate 0.7 Gy/min. Single whole body doses of 4 h Gy and 6.2 Gy were given in the morning. CAP-chloramphenicol, soluble (10%), SPOFA

(chloramphenicolum succinicum natrium) was injected subcutaneously every eight hours in doses of 320 mg of base per 1 kg of body weight in volumes of 0.2 ml. Control animals were injected equivalent volumes of water for injection. A 5 day (15 injections, the last one given in the evening) and 3-day treatment (10 injections, the last one given in the morning) were chosen of which the former was started 5 days, the latter 7 days after irradiation. In normal unirradiated mice the serum levels of active CAP were determined by the method of LEVINE & FISCHBACH (1951) 4 hours after the first subcutaneous dose of 320 mg/kg of body weight. An average serum concentration of 14.6 µg/ml was found.

Determination of peripheral blood cells. Blood samples were drawn from a fine incision in the tail vein. The total red and white blood cell counts were determined using a Coulter Counter apparatus. Repeated blood withdrawals were made which made it possible to express changes in individual values with respect to the initial state.

⁵⁹Fe incorporation into the heme of erythropoietic organs. ⁵⁹Fe citrate (Rotop G D R) diluted with physiologic saline with the addition of benzyl alcohol 0.9% and 1 p.p. injected in an amount of 3.7 × 10⁴ Bq was used. After 6 h the animals were killed by cervical dislocation, the spleens were excised and the skeletons were cleared of the soft tissues by placing into a colony of the saprophagous insect *Der*

mestes vulpinus Fbr. Extraction of heme iron from the spleen and the entire skeleton was carried out by an original method (VACHA et coll. 1978) with the aid of acid ethylacetate. Radioactivity of the extracts was measured using the Nuclear Chicago Automatic Gamma Well Counting System and expressed in per cent of the activity applied.

Bone marrow cellularity. Bone marrow of femoral diaphyses was washed with isotonic serum and suspended. From a part of the suspension a smear was prepared which was differentiated after staining with May Grunwald and Giemsa Romanowsky stains. The rest of the suspension served for determination of the number of nucleated cells in Burker's chamber. Erythropoiesis was represented by proerythroblasts and by basophilic polychromatophilic and orthochromatic erythroblasts. Granulopoiesis included all mature and immature forms of eosinophilic, basophilic and neutrophilic granulocytes. Nucleated elements included cells of the erythroid and granulocytic line and moreover lymphocytes, megakaryocytes, plasma cells, monocytes, reticular cells and unidentified cells.

Spleen colony forming cells (CFU-S). The method of TILL & MCCULLOCH (1961) was used. A pool of tibial bone marrow cells was prepared by standard procedure and the recipients injected different amounts (3×10^4 to 2×10^5) of cells 3 h after irradiation with 6.2 Gy. On day 10 after irradiation the surface splenic colonies were counted after fixation in Bouin's solution. After subtracting the background of endogenous colonies, the number of colonies per bone marrow in one tibia was determined.

Autoradiography. Bone marrow of the femurs was treated by standard procedure (BENFIS & DRÁŠIL 1962). Cell suspensions were labelled with 7.4×10^4 Bq ^3H dTh (per 10^7 cells) at 37°C for 1 hour. Autoradiography using the stripping film technique (Kodak AR 10) was carried out from the smears. The labelling index (number of labelled cells per 1000 observed cells) characterizing the number of cells in S phase of the cell cycle was evaluated. A labelled cell was determined with 99 per cent probability. Labelled cells of the red line are represented by proerythroblasts, basophilic and polychromatophilic erythroblasts. Granulopoiesis included myeloblasts, promyelocytes and myelocytes.

Statistics. The Student's *t* test and the chi square test were used. The values given in the data represent the mean \pm SE.

Table 1

⁵⁹Fe incorporation into heme of spleen and bone marrow of whole skeleton in control and CAP treated mice 10 days after 4.8 Gy (6-8 animals per group used)

	10 days after irradiation		20 days after irradiation	
	Spleen	Bone marrow	Spleen	Bone marrow
4.8 Gy	0.84 \pm 0.17	7.00 \pm 0.64	18.04 \pm 1.76	18.14 \pm 1.66
4.8 Gy + CAP	0.77 \pm 0.05	3.00 \pm 0.36	17.90 \pm 1.64	4.4

* Difference from control $p < 0.01$

Results

An obvious evidence of CAP toxicity in the irradiated organism is provided by the rate of survival of irradiated animals (Fig. 1). A 5 day CAP treatment resulted in a significant ($p < 0.01$) increase in the 30 day mortality after 6.2 Gy (75% compared with 20% in controls). No mortality occurred in animals irradiated with 4.8 Gy and treated with the employed CAP doses. Effects of CAP on hemopoiesis were further examined using this sublethal dose.

Results given in Table 1 demonstrate a depressed influence of the 5 day treatment with CAP upon ^{59}Fe incorporation into the heme of spleen and bone marrow at the end of the treatment period, i.e. 10 days after irradiation. Twenty days after irradiation differences were no longer observed, which suggests reparability of CAP induced depression of the heme synthesis. Whereas ^{59}Fe incorporation into the heme of the bone marrow remained at this postirradiation period approximately on a normal level, ^{59}Fe incorporation into the heme of the spleen exhibited a overshoot (the normal level of ^{59}Fe incorporation into splenic heme amounting to about 2.1%) comparable to the findings on the dynamics of postirradiation recovery of erythropoiesis (BRADY et al. 1976).

Peripheral leukocyte and erythrocyte counts were determined in irradiated animals after a 3-day CAP treatment. The effects of CAP on erythrocyte counts are shown in Fig. 2 as per cent change against the values determined on day 6 after irradiation before starting CAP treatment. A transient increase in peripheral erythrocyte counts is apparent in the CAP treated group on day 14 after irradiation.

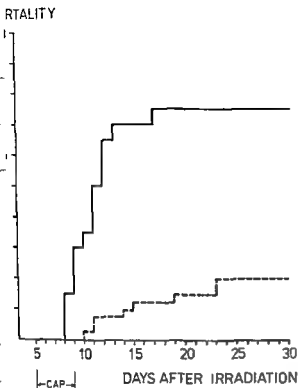


Fig. 1. Cumulative mortality (in per cent) in 40 control (—) and 70 irradiated (---) mice after 6.2 Gy.

rate of recovery beginning 17 days after irradiation does not seem to be affected. The leukocyte counts in the same experiment are given in Fig. 3. The results are expressed by means of a factor of increase, i.e. the ratio of leukocyte counts at a given day to the leukocyte counts before CAP treatment on day 8 after irradiation (average value 860 mm^3). In the CAP treated animals a lower rate of leukocyte count recovery is obvious, which is indicated by decreased values observed on day 10.

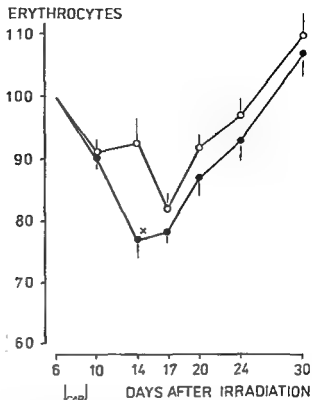


Fig. 2. Per cent of erythrocyte counts in control (O) and CAP treated (●) mice at various intervals after 4.8 Gy (10 animals per point used). x difference from controls $p < 0.05$.

after irradiation and by a lower level of leukocyte counts at other intervals of postirradiation recovery.

A 3 day CAP treatment was chosen to analyse the effect of the antibiotic on the cytology and content of CFU S in the bone marrow of normal and irradiated mice. Bone marrow samples were drawn 4 h following the last injection of CAP, that is 10 days after 4.8 Gy in experiments with irradiated mice. As expected, 10 days after irradiation bone marrow cellularity and number of CFU S were decreased (Ta-

Table 2

Number of cells of the main bone marrow populations (in thousands per femur) and of CFU S per tibia in non irradiated and irradiated (10 days after 4.8 Gy) control and CAP treated mice (for the graphic representation of the differential count 3 animals per group for CFU S donors and 10 recipients per group used).

	Non irradiated	Non irradiated + CAP	4.8 Gy	4.8 Gy + CAP
Erythropoiesis (total)	748 ± 463	945 ± 111	465 ± 67	244 ± 65
Granulocytopenia (total)	7976 ± 168	1507 ± 85	365 ± 168	252 ± 89
Lymphocytes	939 ± 34	7067 ± 00	39 ± 101	186 ± 45
Nucleated elements	10467 ± 77	6400 ± 51	1983 ± 60	1467 ± 394
CFU S	133 ± 48	909 ± 41	77 ± 1	50 ± 6

Difference from controls $p < 0.01$

ble 2) The total cellularity as well as cells of the erythroid granulopoietic and lymphoid lines were decreased after CAP treatment both in control and irradiated animals. Due to the small number of mice used, no statistic evaluation of these results was made. The amount of CFU S in the tibia was however significantly decreased in the CAP treated groups and reached its lowest level with the irradiated and CAP treated animals.

Analogous conditions of 3 dry CAP treatment were employed to analyse cytokinetic characteristics of femoral bone marrow by autoradiography (Table 3). Irradiation per se reduces in a given interval the number of white and red blood cells in the S phase. However the differences are not statistically significant. A recovery of postirradiation mitotic depression can already be anticipated 10 days after sublethal irradiation. However CAP decreased significantly cellular proliferation in both unirradiated and irradiated animals, in either case more markedly with the erythroid line. The numbers of marrow cells entering S phase were lowest with the irradiated and CAP treated group.

Discussion

The CAP dose used corresponds with respect to the serum concentration achieved to clinically employed doses designated as high ones (BARTMANN 1974). According to SUHLAND & WEISBERGER (1963) erythropoietic disorders are to be anticipated when serum concentrations of CAP higher than 15 µg/ml are reached. These calculations do not take into consideration possible changes of the pharmacokinetics of CAP in the irradiated organism. A significant affection of the liver and kidney functions, which might lead to persistence in the blood of higher CAP values and thus to more marked manifestations of its toxicity (SUHLAND & WEISBERGER) cannot however be expected under the acute conditions following the radiation doses used.

The finding of an increased postirradiation lethality after CAP treatment may be considered as the result of an additive action of radiation and CAP on the hemopoietic functions. The action of CAP is manifested by a transiently lower effectiveness of production of the red and white blood elements and its cause is evidently also the depressive action of CAP at the level of the stem cells.

FIRKIN et coll. injected normal mice with higher

FACTOR OF INCREASE (Le)

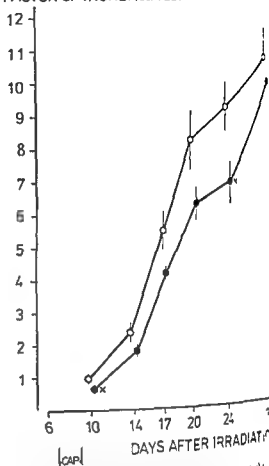


Fig. 3 Factor of increase of leukocyte counts in control and CAP treated (●) mice at various intervals after 4.8 Gy (10% per point used). × difference from controls $p < 0.05$.

Table 3

Number of femoral cells in S phase in non irradiated and irradiated (10 days after 4.8 Gy) control and CAP treated mice as per cent of non irradiated control group (5-10 autoradiograph evaluations per group used).

	Non irradiated	4.8 Gy
Controls		
Erythropoiesis	100	71.0
Granulocytopoiesis	100	81.8
CAP treated		
Erythropoiesis	41.8*	37.4
Granulocytopoiesis	74.7	67.5

Difference from controls
Difference between erythropoiesis and granulocytopoiesis $p < 0.01$

Doses maintaining blood levels of 20 to 40 $\mu\text{g/l}$ for 5 days and observed reduction of reticulo-endothelial cells in the blood decreased numbers of nucleated cells in the marrow and an increase in splenic colony forming units (CFU S) and in the agar colony forming units (CFU C). This effect was explained by inhibition of CFU S and CFU C in the bone marrow with a lowered degree of their differentiation to mature cell forms. The disagreement of the present results with those of FIRKIN et coll may be due to different experimental conditions and by differences in the dynamics and distribution of stem cell compartments in various strains of mice. An inhibitory action of CAP in therapeutic doses upon the growth of human and mouse marrow cells in agar cultures was demonstrated by MORLEY et coll (1974). With the use of techniques for in vitro examination of the growth of mouse and human erythroid (CFU E) and granulocytic (CFU G) colony forming units YUNIS and ADAMSON (1977) demonstrated increased vulnerability of the erythroid stem cells to CAP. In agreement with these findings are also the present results showing a more marked inhibition of proliferation of marrow cells of the red line during CAP treatment in mice. These effects are apparently related with the clinical experience that suppression of erythropoiesis from large doses of CAP occurs with much greater regularity than suppression of granulopoiesis (YUNIS).

The suppression of hemopoiesis by CAP probably results from the inhibition of mitochondrial protein synthesis (MARTELO et coll 1969 NIJHOF et coll 1977) which must ultimately be reflected also in the depression of cellular proliferation and differentiation. Particularly the rapidly dividing cells and tissues are thus prone to side effects of this antibiotic. The results presented show that these effects may adversely affect recovery processes in hemopoietic cell populations of the irradiated organism and corroborate the assumption that radiation syndrome is a contraindication for the therapeutic use of CAP.

SUMMARY

The effect of chloramphenicol given in high therapeutic doses in the course of postirradiation syndrome in

irradiated mice of C₃H/10 strain is reported. Chloramphenicol treatment increased postirradiation mortality of animals and intensified suppression of the proliferating and differentiating processes in hemopoietic organs of the irradiated animals. The results support the assumption that postirradiation depression of hemopoiesis is a contraindication to the therapeutic employment of this antibiotic.

REFERENCES

- BARTMANN K. Antimikrobielle Chemotherapie. Springer Verlag Berlin Heidelberg New York 1974.
- BENES L. and DRASIL V. Incorporation of formate 14C and orthophosphate 32P in bone marrow cells in vitro. I. Effect of cytosine deoxyriboside 5' phosphate. Fol Biol (Praha) 11 (1962) 40.
- BRADY L. W., MARKOE A. M., RUGGIERI S. and BRODSKY I. The effect of sublethal x irradiation of erythropoiesis in the mouse. Int J Radiat Oncol Biol Phys 1 (1976) 471.
- CRONKITE E. P. and BOND V. P. Radiation injury in man. Its chemical and biological basis. Pathogenesis and therapy. Charles C. Thomas Springfield Illinois 1960.
- FIRKIN F. C., SUMNER M. A. and BRADLEY T. R. The influence of chloramphenicol on the bone marrow hemopoietic stem cell compartment. Exp Hemat 2 (1974) 264.
- LEVINE J. and FISCHBACH H. The chemical determination of chloramphenicol in biological materials. Antibiot Chemother 1 (1951) 59.
- MARTELO O. J., MANYAN D. R., SMITH V. S. and YUNIS A. A. Chloramphenicol and bone marrow mitochondria. J Lab Clin Med 74 (1969) 927.
- MORLEY A., FURNESS M. and HIGGS D. Inhibition of growth of marrow cells by chloramphenicol. Aust J Exp Biol Med Sci 52 (1974) 847.
- NIJHOF W., WIERENGA P. K. and KARDAUN S. The effect of thiampenicol on the production of immature red blood cells under anaemic conditions. Brit J Haemat 36 (1977) 29.
- SUHRLAND L. G. and WEISBERGER A. S. Chloramphenicol toxicity in liver and renal disease. Arch Intern Med 112 (1963) 747.
- TILL J. E. and McCULLOCH E. A. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 14 (1961) 213.
- VACHA J., DUNGEL J. and KLENWACHTER V. Determination of heme and non heme iron content of mouse erythropoietic organs. Exp Hemat 6 (1978) 718.
- YUNIS A. A. Drug induced bone marrow injury. Adv Intern Med 15 (1969) 357.
- and ADAMSON J. W. Differential in vitro sensitivity of marrow erythroid and granulocytic colony forming cells to chloramphenicol. Amer J Hemat 2 (1977) 355.

COLLIMATION OF HIGH ENERGY ELECTRON BEAMS

I LAX and A BRAHME

The principal aim in the design of high energy electron beams for radiation therapy is to obtain broad uniform beams of well defined energy and direction. Of equal importance is the collimation of the beam in order to limit its size and to protect surrounding healthy tissues. Even if the electron beam flattening system is almost ideal producing a plane parallel and monoenergetic beam, the collimator will necessarily degrade the quality of the beam to some extent. This degradation will be greater for smaller field sizes, since the fraction of collimator scattered electrons becomes of increasing importance. Therefore, the purpose was to optimize the collimation geometry and the choice of collimator materials in order to obtain a minimum contribution of scattered electrons in the therapeutic beam (BRAHME 1978).

Several reports on the effect of electron scatter near a discontinuity have appeared due to its importance in dose planning (BREITLING & VOGEL 1963, KARJALAINEN et al. 1968, POHLIT 1969, MANDOUR & HARDER 1978, GOITEIN 1978). However, no systematic analysis of the electron scatter at a collimator edge where electrons are completely stopped seems to have been published. This problem has particular implications in the use of small field sizes since electrons scattered from the collimator can contribute considerably to the absorbed dose and perturb the dose distribution of a beam of otherwise high quality. The effect of small energy electron scatter on the angular spread

The field size dependence of the central axis depth dose distribution has been investigated extensively (MARKUS 1960, V. D. DECKERT 1956). The

reduced build up and loss of therapeutic range for small field sizes is due to two different effects which previously have not been clearly distinguished experimentally. It will be demonstrated that the influences of both these effects, namely that of the collimator scattered electrons and that of the field size itself on the dose distribution depends on the lateral motion of electrons in the materials of the collimator and the phantom, respectively.

Theory

General considerations Four principal phenomena are involved when broad electron beams are collimated in order to produce therapeutic electron beams of highest possible quality and consequently smallest possible beam contamination.

First, the presence of air is of great importance particularly at energies below 15 to 20 MeV, since it greatly influences the paths of the electrons due to the appreciable scattering power of the air at these energies (Fig. 1 ray No. 1). This problem has been treated in detail by BRAHME (1971, 1977) and it can conveniently be overcome by the balloon type of collimation.

Secondly, the collimator wall which ideally should be parallel to the direction of the electron paths in the beam will generally scatter slightly degraded electrons back into the beam (BRIOT et al. 1973, Fig. 1 ray No. 2). This phenomenon has been analysed extensively by MANDOUR & HARDER (1975) and MANDOUR (1978). It is of less

importance when the collimator and the effective electron source are well aligned and the effective source size is small so that the electrons reflected almost at glancing incidence will lose very little energy remaining almost indistinguishable from the primary beam.

However when the collimator walls are not parallel to the electron paths they may contribute considerably to the angular spread of the electrons reaching the irradiated surface (BRAHME 1978). This will increase the absorbed dose at the surface and displace the point of maximum absorbed dose closer to the surface since the electrons more rapidly reach a state of full diffusion (BRAHME 1978). This effect has recently been observed experimentally (VAN DER LAARSE *et al.* 1978). It is only to a small degree due to the energy degradation of the electrons scattered from the collimator (cf. MANDOUR & HARDER 1975).

The third type of contamination is due to the production of bremsstrahlung in collimators or apertures (Fig. 1 ray No. 3) and has also been treated extensively (WIDEROE 1959; GIARRATANO *et al.* 1975). In principle it can be overcome by using low atomic number collimators followed by high atomic number photon absorbers.

The last type of contamination which will be reported here in more detail is often of the greatest importance for the dose distribution particularly in the build up region. This contamination is due to the large number of electrons which enter the collimator on the source side and are not stopped but scattered out into the beam through the collimator edge (Fig. 1 ray No. 4).

Angular distribution. It has been shown that in the Gaussian approximation the spherical fluence of primary electrons at a depth z in a medium is according to BRAHME (1975) given by

$$\Phi(\theta_x) = \frac{\exp\{\theta_x^2/\bar{\theta}^2\}}{(\pi\bar{\theta}^2)^{1/2} \cos \theta_x} \quad (1)$$

where θ_x is the projected angle of the electrons on the r - z plane and $\bar{\theta}^2$ is the mean square scattering angle of the electrons at a depth z (Fig. 2). From the spherical fluence the plane fluence in a direction orthogonal to the direction of the incident beam for example in the positive x direction can be calculated

$$N_x(z) = \int_0^\pi \Phi(\theta_x) \sin \theta_x d\theta_x \quad (2)$$

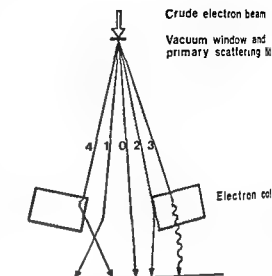


Fig. 1 Representation of the 4 different types of first order contaminations in therapeutic electron beams. Ray No. 1 represents an air scattered electron. No. 2 an electron scattered at the collimator edge. No. 3 an interaction in the collimator where a bremsstrahlung photon is produced and No. 4 an electron scattered out through the collimator edge.

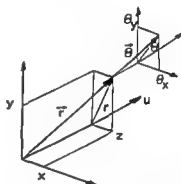


Fig. 2 Coordinate system used to describe the lateral and angular distribution of electrons in an electron beam.

If the mean square scattering angle $\bar{\theta}^2$ is less than 1 the integral in eq. (2) can be developed in a power series. When this is the case the unidirectional plane fluence along the x -axis takes the form

$$N_x(z) = \left(\frac{\bar{\theta}^2}{4\pi}\right)^{1/2} \left\{ 1 + \frac{1}{2} \bar{\theta}^2 + \frac{1}{6} \bar{\theta}^4 + \dots \right\}$$

The mean square angle of scattering at the depth z is according to BRAHME (1975) given by

$$\bar{\theta}^2 = \bar{\theta}_0^2 + T \left(1 - \frac{S_{11}}{E_p}\right)$$

where $\bar{\theta}_0^2$ is the mean square angle of the incident beam at $z=0$, T is the linear scattering power

Table 1
The diffusion depth \sim mm for different materials and energies

Material	H O	C H	C	Al	Cu	Sn	W	Pb	U
E_p/MeV									
5	15.2	16.3	10.9	3.4	0.97	0.85	0.24	0.38	0.71
10	34.0	35.6	23.6	17.0	2.48	2.76	0.66	1.05	0.60
20	68.9	71.4	46.8	74.7	5.37	5.06	1.52	2.43	1.40
30	98.3	102.1	66.4	34.7	7.50	7.06	2.17	3.46	2.00
40	123.0	118.4	82.7	47.4	8.98	8.36	2.56	4.14	2.39
$\rho/\text{g cm}^{-3}$	1	1.05	1.73	2.69	8.92	7.31	19.3	11.3	18.7
$D/D_{\text{H O}}$	1.00	0.95	0.66	0.44	0.13	0.14	0.047	0.081	0.048
eq (16)					Brass				
10 MeV					0.14				
$D/D_{\text{H}_2\text{O}}$		0.95		0.44	0.16		0.05	0.09	
experimental									
10.5 MeV									

which is obtained by multiplying the mass scattering power with the density (BRAHME 1972; ICRU 1972).

The factor within the parentheses (which corrects for the increase in scattering power with increasing depth) contains the total stopping power S_{tot} of the medium and the most probable electron energy E_p of the mean unidirectional plane fluence along the x -axis over the depth interval of the practical range R_p can be evaluated by integrating eq. (3) after insertion of eq. (4).

$$N = \frac{\int_0^R N_x(x) dx}{R_p} = \left(\frac{TR_p}{9\pi} \right)^{1/2} \left\{ 1 + \frac{\bar{\theta}_0}{TR_p} \left(\frac{1}{2} - \bar{\theta}_0^{1/2} \right) + \dots \right\} \quad (5)$$

In order to analyse the material and energy dependence of eq. (5) a first approximation may be assumed that $R_p = kE_p$ with $k = 0.5 \text{ g cm}^{-2} \text{ MeV}^{-1}$ independent of material and $T = (E_p/E_0) \lambda_0^{-1}$ where $\lambda_0 = 1.2 \text{ MeV}$ and X_0 is the radiation length according to ROSS (1952). If it is further assumed that $\bar{\theta}_0 = 0$ eq. (5) appears as

$$N_x \approx \left(\frac{kE_p^2}{9\pi} \right)^{1/2} \times (E_p \lambda_0)^{-1/2} \quad (6)$$

Thus the mean unidirectional electron fluence in a narrow Gaussian beam decreases with energy but increases with atomic number since the radiation length is inversely proportional to the atomic number. It should be pointed out that the eqs. (5) and (6) are slight overestimations since the angular

spread remains approximately constant at depths beyond the diffusion depth (cf. eq. 13).

Energy distribution Since the electrons are scattered more and more laterally at increasing depths the mean energy of the electrons leaving the collimator edge is considerably lower than the incident energy. If it is assumed that the mean energy of the electrons decreases linearly with depth according to $E(x) = E_0[1 - (x/R_p)]$ the mean energy of the laterally scattered electrons \bar{E} can be obtained by evaluation of

$$\bar{E} = \frac{\int_0^R E(x) N_x(x) dx}{\int_0^R N_x(x) dx} \quad (7)$$

which after insertion of eq. (3) reduces to

$$\bar{E} = 0.4 E_0 \left\{ 1 + \frac{1}{2} \left(\frac{\bar{\theta}_0}{TR_p} \right) + \dots \right\} \quad (8)$$

The mean energy of the laterally scattered electrons is thus approximately 40 per cent of the incident mean energy regardless of the material when the mean square scattering angle of the incident electrons is small.

Lateral electron displacement In order to obtain a complete description of the total number of low energy electrons scattered out through a collimator edge the lateral movement of the electrons must also be considered. When a plate collimator is in

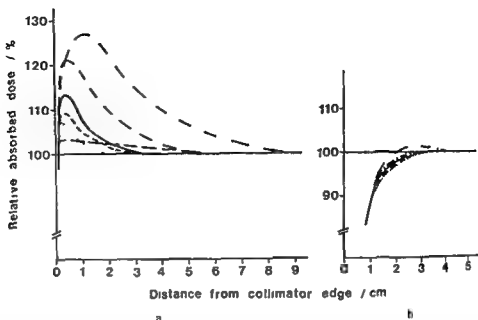


Fig 3 a) Relative absorbed dose in water due to electrons scattered out through a collimator edge. These and consecutive curves show the ratio of absorbed dose in the field with and without collimator in place normalized to 100 per cent in the non collimated field. The energy (E) = 10.5 MeV and the depth of measurement 1 mm. Zero point of abscissa is the point where exactly half the detector is covered by the shadow of the col-

limator. Relative absorbed dose in this point is close to 40 per cent. b) Same as in (a) but depth of measurement 30 mm. Comparison between (a) and (b) clearly demonstrates that almost all the collimator scattered electrons are stopped in the build up region (cf Fig 7). — CH — Al — W — Pb — — Al + 1 mm Pb

radiated a maximum distance exists from the edge of the collimator beyond which electrons no longer can be scattered laterally and escape at the edge. This distance of maximum lateral displacement in a given material is also equal to the minimum beam radius necessary to obtain full broad beam conditions at the central axis of a circular beam incident on the material in question. When the simple multiple scattering theory is used the distance of maximum lateral displacement may be approximated by the radius outside which the contribution to the fluence or the absorbed dose at maximum build up is less than one per cent. This can be calculated when the lateral or radial electron fluence is known.

In the Gaussian approximation the lateral displacement of the electrons in an electron beam can according to BRAHME (1975) be expressed by

$$\Phi(r, z) = \frac{\exp\{-r^2/\bar{r}_z^2\}}{\pi r^2} \quad (9)$$

where r is the radial displacement from the z axis, i.e. the central axis of the beam, and \bar{r}_z^2 is the mean square radius of the radial distribution at the depth z (cf Fig 2). The radius R_1 outside which the contribution to the fluence on the central axis is less than one per cent is given by

$$\int_R^\infty \Phi(r, z) 2\pi r dr \leq 0.01 \quad (10)$$

as $\Phi(r, z)$ from eq (9) is normalized to one incident electron at $z=0$. From eq (10) R_1 is easily obtained

$$R_1 = (\bar{r}_z^2 \ln 100)^{1/2} \quad (11)$$

and the mean square radius \bar{r}_z^2 of a narrow beam incident at $z=0$ is according to BRAHME (1975) given by

$$\bar{r}_z^2 = \bar{r}_0^2 z^2 + T_z^2/3 \quad (12)$$

Eqs (11) and (12) should be evaluated at the depth of dose maximum which may be approximated by the diffusion depth (BRAHME 1975) where the electrons have reached a state of full diffusion. For most practical purposes the diffusion depth can be represented by the depth where the root mean square (rms) scattering angle is one radian (BETHE et coll 1938). This depth can be obtained from eq (4) and for a plane parallel beam it is ($\bar{r}_0=0$) given by

$$z_1 = \left(\frac{T}{\rho} + \frac{S_{11}}{E_p \times \rho} \right)^{-1} \rho^{-1} \quad (13)$$

Despite the somewhat arbitrary choice of one radian in the definition of z_1 and the approximate nature of

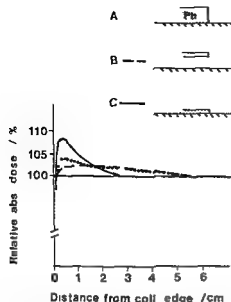


Fig 4 Relative absorbed dose in water due to electrons scattered through a collimator edge of lead. Geometric conditions in the three measurements are shown in the inserted figure. A 25 mm thick lead collimator at surface of water phantom. B 6 mm thick lead collimator 19 mm from the surface. C 6 mm thick lead collimator at surface. Depth of measurement is 1 mm.

z (4) at large depths the diffusion depth given by z (13) is an important transport parameter. It clearly shows that both the scattering and energy loss properties of the medium are involved. By using eqs (12) and (13) eq (11) reduces to

$$R_1 = \left(\frac{\frac{1}{2} \ln 100}{1 + \frac{S_{te}}{E_p T}} \right)^{1/2} \times \sim 1 \quad (14)$$

since the square root factor is fairly close to unity for most collimator materials and energies used in radiation therapy.

As a first approximation z_1 may therefore be used as a measure of the maximum lateral excursion of the electrons. In Table 1 the value of z_1 in mm is given for some representative materials and energies of therapeutic interest. It is quite clear that in high atomic number materials a much smaller region along the collimator edge will be available to scatter electrons at the edge.

Absorbed dose due to edge electrons. The total number of electrons that will be scattered out through the collimator edge will thus depend on the product of two factors which partly compensate each other. First the number of laterally scattered electrons in a narrow beam increases with atomic number due to the increase in scattering power

(cf eq 6). However this factor is normally counter balanced by the second factor which implies that a smaller area along the collimator edge is available to remove electrons laterally in high atomic number materials mainly due to their higher density (cf eq 14). The mean absorbed dose due to electrons scattered out through the collimator edge relative to the absorbed dose in the open field is thus proportional to the product of these two factors

$$D \propto N_x \times \left(\frac{T}{Q} + \frac{S_{te}}{Q \times E_p} \right)^{-1} \rho^{-1} \quad (15)$$

which after inserting T (from ROSSI) and approximating S_{rad} by E_p/V_0 (the correct value at the high energy limit) appears as

$$D \propto \frac{E_p^3 X_0^{1/2} \rho^{-1}}{E_p + E_p X_0 S_{coll} + E_p^2} \quad (16)$$

The absorbed dose due to electrons scattered out through a collimator edge is thus as expected inversely proportional to the density in addition to which it has only a weak dependence on energy and atomic number. In the penultimate row of Table 1 relative values of D are given at 10 MeV normalized to water. It is observed that D_c decreases by a factor of 10 on passing from water to lead. Further more tungsten is almost three times better than brass.

Method and Results

Beam parameters and dosimetry. The experiments were carried out on a linear accelerator MEL 75 10 at one electron energy (E_p) = 10.5 MeV. The energy was determined by range analysis according to NACP (1972). No scattering foil was used during the irradiations in order to reduce the angular spread of the electrons in the beam. The vacuum window, the monitor ion chamber, the mirror for the light beam and the air volume between the phantom and accelerator have been the only scattering materials in the beam giving a field flatness of ± 6 to 7 per cent within 5 cm from the central axis at depths from 1 to 30 mm. The photon beam diaphragms of the machine have been fully opened ($\approx 30 \text{ cm} \times 30 \text{ cm}$) and the distance from the vacuum window to the phantom surface has been 100 cm. The position of the effective electron source was 86 cm from the phantom as determined by ionization chamber measurements at two distances.

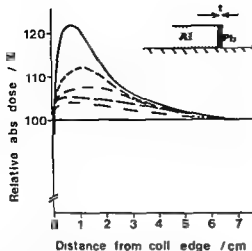


Fig 5 Relative absorbed dose in water due to electrons scattered out through a lead lined collimator edge. Thickness t of lead given in mm and depth of measurement is 1 mm. Thickness of collimator is 25 mm — $t=0$ — 0.10 — 0.22 — 0.44 — 0.6 — ∞

Measurements of the dose distribution were performed with an automatic isodose plotter (Scanditronix RFA 1). The detector was a 2 mm \times 2 mm semiconductor diode. This detector was found to have no significant energy dependence at least between 3.8 and 25 MeV (HULTEN 1975). In the build up region the diode gives values which are too low compared with a liquid ion chamber (HULTEN). The difference could be as much as 5 per cent and is greatest for beams approaching a monoenergetic and monodirectional beam (BRAHME & SVENSSON 1976). The difference is expected to be only 2 to 4 per cent in the present case (BRAHME & SVENSSON) due to the relatively high energy—and angular—spread in the beam mainly caused by the fairly thick monitor ion chamber.

A slit of 1.4 mm width was made in a 3 mm thick tungsten collimator to check the geometric resolution of the detector. A scan was made with the detector across the slit with a distance from the tungsten collimator to the semiconductor element of 2 mm. This gave a FWHM of 2.5 mm at 11.5 MeV as would be expected from the size of the semiconductor element and the slit.

A similar method was used to determine the size of the effective electron source. Instead of the diode film was used as detector and the distance from the tungsten collimator to the film was 50 mm. After correction for multiple air scattering behind the collimator eq. (12) and the width of the slit as well as the size of the aperture of the density reader used, the diameter or more exactly twice

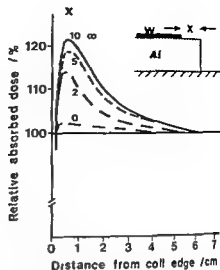


Fig 6 Relative absorbed dose in water due to electrons scattered out through a partly covered collimator. Non-covered distance X in mm. Thickness of aluminum collimator W in mm and of tungsten plate 3 mm. Depth of measurement is 1 mm.

the root mean square radius of the effective electron source was determined to be 45 mm. The angular spread of the beam was thus quite broad and the source size fairly wide though the scatter foil was removed.

Collimator scattering. The absorbed dose due to electrons scattered out through a collimator edge in water was measured at 1 and 30 mm depth in water for collimators made of polystyrene, aluminium, brass, lead and tungsten (Fig. 3). The collimators had a thickness of 5 to 10 per cent greater than the continuous slowing down approximation range R_{csd} (BERGER & SELTZER 1964) of the electrons in the material giving for polystyrene 55 mm, aluminium 25 mm, brass 80 mm and lead 57 mm at an energy of 10.5 MeV. The mean range R_0 in tungsten is 3.2 mm, but for practical reasons 3.0 mm was used. The collimator was placed in direct contact with the water phantom with the edge of the collimator on the central axis of the electron beam. The dose profile was measured perpendicular to the direction of the collimator edge and the measurement at each point was related to measurement in the uncollimated open field. The effect of the laminated collimator was also evaluated using an aluminium collimator covered with a lead layer of the edge (Fig. 3a).

The relative values of the mean absorbed dose contribution from collimator scattered electrons normalized to the theoretical value 0.91 for polystyrene (C_6H_8) appear in Table 1. The mean absorbed dose has been estimated by integrating the

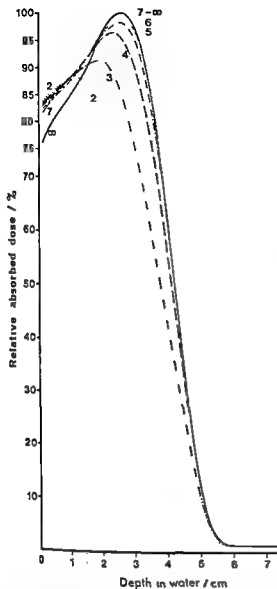


Fig. 7. Central axis depth dose curves for beams with diameters 7 cm and ∞ in steps of 1 cm up to 7 cm and ∞ (30 cm \times 30 cm photon collimators). Curves for 7 cm and ∞ are identical beyond a depth of 1.8 cm. Within this depth a clear difference in relative dose between open and collimated beam due to contribution from electrons scattered out through collimator edge.

area above the 100 per cent level in Fig. 3a. The agreement with the theoretical values from eq. (16) is good. The small discrepancies may be due to uncertainties in the dosimetric techniques and to approximations in the theoretical evaluation.

A series of measurements were performed (Fig. 1b) in order to separate the dose contribution from electrons reflected at the edge (Fig. 1 ray No. 2) and those scattered out through the edge (Fig. 1 ray No. 4). In the first measurement a lead collimator of 25 mm thickness was placed at the sur-

face of the water phantom (A in Fig. 4). In a second measurement a lead collimator of 6 mm thickness was positioned 25–6 = 19 mm from the water phantom (B in Fig. 4). The difference between the two measurements is due to electrons reflected at the surface of the collimator edge from a lead height of 19 mm. The contribution to the absorbed dose from reflected electrons is thus for most purposes negligible in comparison with the contribution from electrons scattered out through the collimator edge. It can also be seen in Fig. 4 that the reflexion angle is small as expected (MANDOUR & HARDER 1975) when the collimator edge is well aligned with the electron source. The curve for a 6 mm lead collimator placed at the surface of the water phantom is illustrated in C in Fig. 4. This clearly demonstrates the difference in dose distribution caused by different geometries. In cases B and C the same number of electrons is scattered out and their angular distribution is the same.

Due to the greater distance from the surface in case B the electrons are distributed over a larger area. The geometric effect mentioned explains part of the favorable effect obtained when a high atomic number lining is added to a low atomic number collimator (Fig. 3a). Alternatively it is possible to have a few cm of clearance between the last collimator and the surface to avoid the hot spots near the edge of the collimator (Fig. 3). However the laminated collimator is the best choice at high energies when the bremsstrahlung production must be minimized. The effect of different lead thicknesses on an aluminium collimator was measured and the results are given in Fig. 5. It may be concluded that a thickness of about 0.5–1 mm is for practical purposes sufficient to remove most of the scattered electrons from the aluminium collimator. The use of tungsten instead of lead would be even more effective (Table 1) and a thickness of 1.3 mm W (0.5–1) would be enough for an energy of 40 MeV.

The contributions of different lateral layers (X in Fig. 6) in an aluminium collimator to the number of electrons scattered out of the edge were evaluated in a separate measurement illustrated in Fig. 6. The most laterally displaced electrons give the smallest contribution to the absorbed dose in the beam. About 90 per cent of the electrons scattered out through the edge originate within 0.5–1 mm from the edge of the collimator.

Field size dependence. An aluminium collimator covered with 1 mm of lead at the edge gives an

additional dose at the edge of the field from collimator scattered electrons of only a few per cent in a broad beam (Fig. 3a). This type of collimator has therefore been used to measure the field size dependence of the depth absorbed dose curve.

The diameter of the circular diaphragms in the collimator plates ranged from 20 mm up to 70 mm in steps of 10 mm; the thickness of the collimators was 25 mm. Again the collimator was placed in direct contact with the surface of the water phantom concentric with the central axis of the beam. The relative depth dose curve was measured on the central axis of the collimated beam.

The results of the measurements appear in Fig. 7. The curves are normalized to 100 per cent at dose maximum in the open beam. The precision in the measured curves is about one per cent.

A number of important conclusions can immediately be drawn from these results. The mean energy of the electrons scattered out of the edge is only about 40 per cent of the mean energy of the incident electrons (eq. (8)). This means that only the build up region is influenced by the collimator scattered electrons; this is evident in Figs 7 and 3b. The dose at one mm depth is increased from 76 per cent to 82 per cent when the beam is collimated from the open field to 7 cm diameter. For depths of more than 18 mm the two curves are almost identical since most of the collimator scattered electrons are lost. The use of a low density collimator may therefore be a valid method for increasing the surface dose for the treatment of small superficial lesions.

The general shape of the narrow beam curves agree well with the recent Monte Carlo results of BERGER & SELTZER (1978). It is evident that the practical range no longer is suitable for energy determination when the beam cross section is much smaller than z_1 since the usual energy range relation is violated.

The absorbed dose at the depth of dose maximum in a collimated beam is compared with that of an open beam in Table 2. It can be seen that the absorbed dose has decreased by one per cent at a field cross section of 6 cm. This is in fair agreement with the approximate value $2z_1$ as deduced in eq. (14). The value of z_1 is 7 cm for water at 10 MeV.

The value of z_1 in Table 1 can thus be used as the minimum radius for obtaining broad beam conditions in a uniform beam.

Table 2

Field size dependence of the maximum absorbed dose at 10.5 MeV

Field size (cm)	Relative absorbed dose (per cent)
Open field (30×30)	100
Ø 7	100
Ø 6	99
Ø 5	98
Ø 4	96
Ø 3	91
Ø 2	88

Conclusion

Electrons which enter a collimator on the side and are scattered out through the collimator edge are often the most important form of beam contamination in electron beams. They can add significant doses to the build up region when low density collimators are used. This effect is minor for high density collimators separated from the patient by a few cm or which is even better from the photon contamination point of view a low atomic number collimator with a high density layer having a thickness equal to half the diffusion depth in the material in question.

It is demonstrated that the diffusion depth is the relevant parameter for comparison with the field size to evaluate whether broad beam dose distributions are obtained on the central axis of the beam. Furthermore it may be concluded that the use of the collimator to improve the uniformity of an insufficiently flattened electron beam is a very poor method since it has a negligible effect from the dose maximum down to the therapeutic range. However, on such accelerators it is of little value removing the collimator scattered electrons without simultaneously improving the beam flattening since the scattered electrons do contribute somewhat to the uniformity in the superficial layers, particularly when the collimator is in direct contact with the skin.

SUMMARY

The collimation of high energy electron beams for radiation therapy is treated with special attention on the contamination of the beam by electrons which are scattered out of the beam through the edge of the collimator. It is demonstrated that the

lated that the mean energy of these electrons is only per cent of the mean energy in the beam and that smallest electron contamination is obtained if the enal closest to the beam is of high density

ACKNOWLEDGEMENT

any valuable suggestions of Lars Eric Larsson Hans nsson and Rune Walstam are gratefully acknowledged t of this investigation was supported by grants from g Gustaf V Jubilee Fund

REFERENCES

- IGER M J and SELTZER S M Tables of energy losses and ranges of electrons and positrons NASA SP 3017 1964
- National Bureau of Standard Int report NBSIR 78 1552 1978
- RHE H A ROSE M E and SMITH L P The multiple scattering of electrons Proc Amer Phil Soc 78 (1938) 573
- ANKE A Multiple scattering of relativistic electrons in air Div Electron Phys Royal Inst Tech Stockholm Sweden Int report Trita EPP 71 22 1971
- On the optimal choice of scattering foils for electron therapy Div Electron Phys Royal Inst Tech Stockholm Sweden Int report Trita EPP 72 17 1972
- Simple relations for the penetration of high energy electron beams in matter Nat Inst Rad Prot Stockholm Sweden SSI 1975-011
- Electron transport phenomena and absorbed dose distributions in therapeutic electron beams Livro de resumos Abstract No S 0340 p 198 Fourteenth International Congress on Radiology Rio de Janeiro 1977
- Physical aspects on equipment for external beam radiation therapy Precision demands in external radiation therapy Proceedings of a symposium Örebro Sweden May 1978
- and SVENSSON H Depth absorbed dose distributions for electrons Phys in Med Biol 21 (1976) 304
- EITLING G und VOGEL K H Dosisverteilung bei der Bestrahlung inhomogener Medien mit schnellen Elektronen Strahlentherapie 122 (1963) 321
- LOT E DUTREIX A DUTREIX J et PENET A Etude expérimentale de la collimation des faisceaux d'électrons par un diaphragme de plomb réglable J Radiol Electron 54 (1973) 39
- D DECKEN C ■ Tiefendosiskurven bei der Bestrahlung mit schnellen Elektronen in Abhängigkeit von der Energie und der Fiedgrosse Strahlentherapie 101 (1956) 204
- GIARRATANO J C DUERKES R J and ALMOND P R Lead shielding thickness for dose reduction of 7 to 28 MeV electrons Med Phys 6 (1975) 336
- GORTEN M A technique for calculating the influence of thin inhomogeneities on charged particle beams Med Phys 5 (1978) 258
- HULTÉN G A comparison between absorbed dose distributions measured with Scanditronix radiation field analyzer RFA 1 and conventional methods Proceeding of NACP meeting Reykjavik Iceland 1975
- ICRU 21 Radiation Dosimetry Electrons with initial energies between 1 and 50 MeV 1972
- KARJALAINEN P BRENNER M and RYTILÄ A Effect of anatomical irregularities on the dose in electron beam therapy Acta radiol Ther Phys Biol 7 (1968) 129
- VAN DER LAARSE R BRUINVIS I A D and FARIP NOONAN M Wall scattering effects in electron beam collimation Acta radiol Oncology 17 (1978) 113
- MANDOLR M A Analyse des Durchgangs energiereicher Elektronen durch räumlich begrenzte und inhomogene Medien mit Hilfe der Monte Carlo Methode Thesis University of Würzburg 1978
- und HARDER D Scheinbare Reflexion schneller Elektronen bei streifendem Einfall Z Naturforsch 30 (1975) 265
- — Berechnung der Dosisverteilung schneller Elektronen in und hinter Gewebelinien mit beliebiger Breite Strahlentherapie 154 (1978) 546
- MARKUS ■ Dosisverteilung schneller Elektronen zwischen 3 und 15 MeV und ihre Beeinflussung durch Herdblenden und Tubusse Strahlentherapie 112 (1960) 322
- Nordic Association of Clinical Physics (NACP) Procedures in radiation therapy dosimetry with 5 to 50 MeV electrons and roentgen and gamma rays with maximum photon energies between 1 MeV and 50 MeV Acta radiol Ther Phys Biol 11 (1972) 603
- POHLIT W Calculated and measured dose distributions in inhomogeneous materials and in patients Ann N Y Acad Sci 161 (1969) 189
- ROSSI B B High energy particles p 67 Prentice Hall New York 1952
- WIDEROE R Measurement problems in high energy electron and X ray therapy with a 31 MeV betatron In Quantities units and measuring methods of ionizing radiation p 251 Edited by F Fossati U Hoepli Milan 1959

MICRODOSIMETRY

II Use of secondary electron emission to simulate two target models

T E BURLIN and B J FORSBERG

Secondary electron emission has been suggested as a phenomenon offering considerable potential for microdosimetric analyses (BURLIN 1974). Previous exploration of secondary electron emission as an alternative technique for microdosimetry was reported by FORSBERG & BURLIN (1980) who calculated lineal energy distributions from derived number distributions of secondary electrons (<50 /) from the exit and entry sides of thin tissue equivalent foils bombarded with keV electrons. These distributions can be considered as single volume distributions, i.e. the number of secondary electrons emitted per primary particle was calculated only on one of the sides.

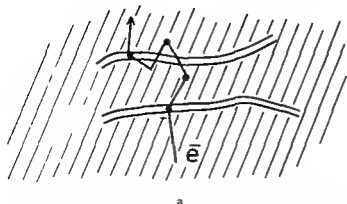
Furthermore measurements of the variance of the secondary electron current have been performed (FORSBERG 1978) and the derived dose mean value lineal energy indicates that the secondary electron emission should be a potential technique for microdosimetry.

In the present report coincident single event distributions from the two surfaces of thin foils of carbon and lithium fluoride are calculated, i.e. the number of secondary electron pairs (n_1, n_2) where n_1 is the number of secondary electrons leaving the incident escape zone and n_2 the number leaving the exit escape zone of the foil simultaneously. These calculations were performed to show the potential of this experimental technique which simulates object size of a few nm separated by up to several

hundreds of nm and could thus be used to investigate the energy deposition in two related volumes.

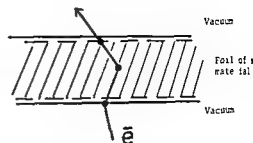
Background Several authors have suggested that some biologic effects may result from energy deposition in two different targets (NEARY 1965, ROSSI 1968, ALPER 1970). NEARY suggested that the targets were in the nanometer region and separated by a distance not exceeding $0.2 \mu\text{m}$. KELLERER & ROSSI (1971) concluded that for cells of higher organism the targets were of nanometer dimensions separated some micrometer. An experimental investigation with proportional counter measurements to the two target theory has been reported previously (BURLIN et al 1972, BENSTOCK et al 1974, 1976). One of the limitations with this technique is the difficulty to simulate target diameters less than $0.5 \mu\text{m}$, two orders of magnitude greater than the size of such a biologic target. In secondary electron emission the relevant volume is a very thin layer the shape would thus be equivalent to a thin membrane which has been proposed by ALPER as a sensitive structure in her two target theory. ALPER suggested that damage from radiation in non nucleic acid sites can be identified as lesions in membranes. A cell death could then occur if DNA or an enzyme was attached to a particular site on the membrane and an interaction took place between the two volumes.

The cell membranes vary in thickness due to if



a

Fig 1 a) Two biologic targets which can be simulated by the experimental arrangement shown in (b) b) Thin foil in vacuum



b

irradiated by electrons. The dotted lines indicate the escape zones at the incident and exit side

they consist of a single layer then approximately 10 nm thick or a multi layer. If several very thin layers exist in the membrane it may be important not only to analyse the stochastic behaviour of energy deposition in one layer but also in two or more associated layers. The shape and thickness of those layers are similar to the escape zones in secondary electron emission. In the present report an alternative technique to twin proportional counter measurements based on the secondary electron emission technique is discussed and some calculations are made to examine the potential of the technique.

Secondary electron emission as an alternative technique for microdosimetry has previously been treated in detail for a single target by FORSBERG & BURLIN.

When a medium is irradiated by ionizing radiation a degradation spectrum is established within the medium. When this electron spectrum passes through the interface between the material and a vacuum some of the electrons will penetrate the potential barrier and escape from the surface with an energy of 0 to 50 eV. These only will be referred to as secondary electrons in the following. The cut off at 50 eV is of course arbitrary but has been widely accepted in the literature on secondary electron emission. The secondary electron spectrum has a marked peak at 1 to 3 eV outside the medium.

The secondary electrons have a very limited range in a solid due to the interaction with the electrons and phonons so that secondary electrons emitted originate from a thin surface layer. This layer from which secondary electrons are able to escape is known as the escape zone. Its thickness is characteristic of the particular material but is independent of the energy of the primary radiation. The

maximum depth of the escape zone is about 10 nm for metal and about 5 times this value for molecules (DIATLOWITSKAJA 1948, NAKHODIN & ROMANOWSKY 1958, BRONSHTEIN & SEAL 1960). Thus secondary electron emission reflects energy depositions within volumes of linear dimensions of approximately 10 nm which is much smaller than can be simulated by the conventional proportional counter technique which is limited to approximately 0.5 μm or more.

If the low energy secondary electrons are recorded at the incident and exit surface related energy depositions in two nm thick volumes separated by a distance approximately equal to the foil thickness can be determined (Fig 1). Fig 1a shows two parallel chromatin targets a few nanometers thick separated by an interaction distance of several tens of nanometers. The probability of one inelastic interaction in one of the targets could be simulated by analysing the energy deposition at the incident and exit escape zone (Fig 1b). In the same way the probability of at least one inelastic interaction occurring simultaneously in both biologic targets can be simulated by determining the probability that each of the two escape zones an inelastic interaction occurs for the same primary particle. All the calculations in the present report are based upon the situation illustrated in Fig 1b.

In traditional microdosimetry much effort has been put into the use of wall less detectors for the reason for this being the so called wall effects (KELLERER 1971). These effects are caused by the different density of the gas and of the walls of the proportional counter. In secondary electron emission hence in the present calculations opposite effects will occur: electrons are coming from vacuum in-

d foil and after interacting in that foil the electron is sometimes will be emitted out to the vacuum. Of three wall effects mentioned by KELER the analogue to the re entry effect will be the most important if the backscattering from polarising electrodes or other material is ignored (Fig. 1). In a homogeneous situation (Fig. 1a) an electron can be backscattered and re enter the sensitive volume and these interactions there. The re entered electron is missed with the secondary electron emission technique unless a backscattering material is introduced. The situation for which the calculations were made will thus underestimate the energy deposited. Other two effects the V effect and the δ ray effect will be small due to the limited range of the δ ray particles for keV electrons used in the calculations.

Simulated object size and target distance The size of the simulated object can usually be derived as follows. The depth of the escape zone is the maximum depth from which the most energetic (50 keV) secondary electrons are able to escape is taken from the literature. The probability of escape decreases with depth and is described by an exponential function. The mean escape depth (or the characteristic escape length) is usually taken as one third of the escape zone. Since in microdosimetry the mean chord length in unit density material is of more interest than the actual dimensions the mean escape length is multiplied with the density of the foil and a constant ($=2$). This constant was taken from BIRKBEFF et coll (1970) who derived the mean chord length for infinite thin slabs irradiated under isotropic conditions. This was used though in the present investigation the primary electrons were directed perpendicular to the foil. This means that the mean chord length given will be an overestimation but it is accurate enough for the present purpose.

The mean chord length at the incident side will always be smaller than at the exit side. The reason for this is that the primary electrons reach the exit surface layer at different angles and a more isotropic situation occurs. This will tend to reduce the energy deposited per event at the incident side compared with the exit side which is also observed in calculated distributions.

The thickness of the escape zones for LiF and C is taken to be 24 and 4 nm respectively. A 100 nm thick LiF foil will thus simulate two unit density volumes with mean chord lengths $2 \times \rho \times (\text{escape zone})/3 = 42$ nm separated by a unit density layer of

approximately $\rho \times d = 250$ nm. Similarly a graphite foil could simulate two unit density volumes with mean chord lengths of 6 nm using a density for carbon of 2.25 g/cm^3 .

Calculations The calculations were based on a Monte Carlo program by McDONALD et coll (1971, 1973) and were described together with necessary input parameters to the program by FORSBERG & BURLIN.

If the calculated probability $P(n)$ that n secondary electrons will emerge per primary electron is multiplied by the average energy expended in the escape zone per secondary electron emitted the probability distribution of lineal energy is obtained.

The two-target situation postulated by several authors referred to in the introduction may also be simulated by extending these calculations. The escape zones at the incident and exit sides of a thin foil simulate the two small volumes (nm) and the thickness of the foil (μm) simulates the interaction distance. The number of secondary electrons released by a single primary electron from both the incident and exit surfaces of the foil was calculated. Each primary electron was incident perpendicular on thin foils of carbon or lithium fluoride with actual thicknesses of maximum $0.1 \mu\text{m}$ and $0.4 \mu\text{m}$ i.e. approximately $0.23 \mu\text{m}$ and $1 \mu\text{m}$ respectively in unit density material. During its passage through the foil the electron will lose energy in discrete steps and produce low energy electrons. Sometimes secondary electrons will emerge from both the incident surface (n_i) and the exit surface (n_e) resulting from energy deposition by the same primary particle (event) giving rise to a coincident event. Registration of the number of secondary electrons in the form (n_i, n_e) including the most common situation $(0, 0)$ ($n_i = 0$) and $(0, n_e)$ was undertaken through an extension of the Monte Carlo program.

Results and Discussion

It was intended to explore the potential of secondary electron emission as a technique for microdosimetry and especially its possible use to obtain information about the energy deposition in two related targets. Only a few examples will be given.

Figs 2 and 3 show $P(n)$ -distributions for emission of secondary electrons at both the incident and exit surfaces. The information obtained may be presented in various ways. It is most probable that a fast electron will pass through the incident escape

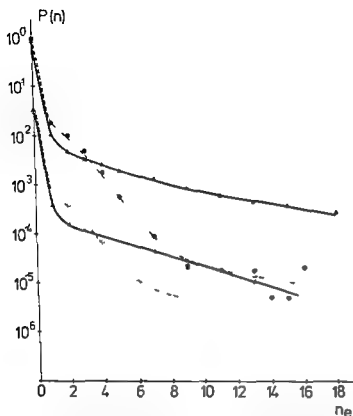


Fig 2 Probability distributions $P(n)$ at the incident and exit surface of a $0.1 \mu\text{m}$ thick carbon foil irradiated by 6 keV electrons. Points show calculated results. Δ Number of secondary electrons emitted from the exit surface when no secondary electrons are emitted from the incident surface $n_i=0$. \bullet Number of secondary electrons emitted from the incident surface when no electrons are emitted from the exit surface $n_e=0$. \triangle Number of secondary electrons emitted from the exit surface when at least one electron is emitted from the incident surface $n_i>0$. \circ Number of secondary electrons emitted from the incident surface when at least one electron is emitted from the exit surface $n_e>0$.

zone without the emission of a secondary electron the probability of releasing 0, 1, 2 etc secondary electrons as it passes through the exit escape zone appears in Fig 2. Such an event would not lead to biologic action involving the two targets unless energy was deposited in the other target by another fast electron before repair processes had intervened. Thus a two target model is simulated when at least one secondary electron is also emitted from the incident escape zone. This is also shown in Fig 2 as the probability that 0, 1, 2 etc secondary electrons are emitted from the exit escape zone when at least one electron is emitted from the incident escape zone. The same figure also shows distributions giving the probability of escape of a secondary electron from the incident escape zone when zero (single volume) and when at least one secondary electron (two targets) are emitted from the exit escape zone. It is evident that the shape of the probability curves is not greatly changed by the consequent requirement

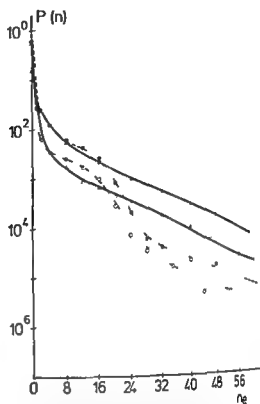
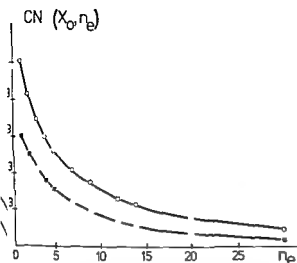


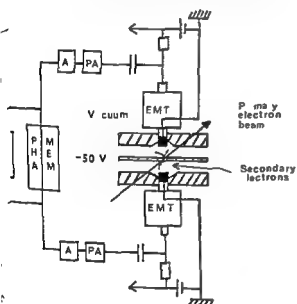
Fig 3 Probability distributions $P(n)$ at the incident and exit surface of a $0.1 \mu\text{m}$ thick lithium fluoride foil irradiated by 6 keV electrons. Points show calculated results. Symbols as in Fig 2.

but the magnitude of probability of an event which can lead to biologic action decreases by about two to three times. As shown in Fig 2 there are two sets of curves with different shapes which depends on whether the secondary electrons (on the average) are emitted from the incident or exit surface. The mean chord length for primary electrons in the incident volume will be smaller than in the exit volume and hence the probability of large numbers of secondary electrons being emitted from the incident side is low compared with the probability on the exit side. The data in Fig 2 relate to a $0.1 \mu\text{m}$ thick carbon foil bombarded with 6 keV electrons. The thickness of the escape zone for carbon is approximately 4 nm . This would simulate in tissue equivalent material two targets with mean chord length about 6 nm separated by some $0.23 \mu\text{m}$.

Similar calculations were undertaken for $1 \mu\text{m}$ thick lithium fluoride foils bombarded with 6 keV electrons (Fig 3). The escape zone thickness for LiF is 4 nm .



4 Number of coincident events for 2×10^6 primary electrons (X_0, n_e) resulting in emission of at least n_e secondary electrons from the exit surface of two lithium fluoride foils irradiated by 6 electrons ■ Thickness = $0.05 \mu\text{m}$ ○ Thickness = $0.1 \mu\text{m}$



5 Experimental arrangement to determine the probability distributions $P(n)$

ately 42 nm. Therefore this simulates in unit density material the situation where two targets with an chord lengths of approximately 42 nm are separated by about $0.25 \mu\text{m}$. The general shape of probability distributions both in the case where a secondary electron is emitted from the incident escape zone when a fast electron passes through and also in the incident escape zone are very similar to the results for carbon.

Figs 2 and 3 together illustrate the effect of different mean chord lengths. The ratio between the sin-

gle and coincident interactions is markedly smaller for LiF than for carbon which is expected because of the longer mean chord length of the primary electrons in the escape zone of LiF.

In Fig. 4 the number per 2×10^6 primary electrons of coincident events when at least n_e secondary electrons are emitted at the exit side is shown at two LiF foil thicknesses i.e. at different target separations. Thus $CN(X_0, n_e) = \sum (n, n_e)$ where $n \neq 0$ and X_0 is the actual foil thickness. For $n = 1$ ($n, n_e \neq 0$) is approximately twice as probable for the thicker foil than for the thinner foil and this probability ratio increases as n_e increases.

Similar calculations for carbon and LiF of approximately the same foil thickness and primary electron energy (6 keV) show that it is about 30 times more usual to get any $(n, n_e) \neq 0$ for LiF than for carbon.

The data presented are the essential information to provide lineal energy distributions. While the calculations have only been undertaken for electrons such calculations can in principle be extended to other ionizing radiations. However that is out of scope of the present report which is aimed at illustrating that an alternative experimental technique exists by which microdosimetric data can be obtained. This technique simulates target sizes in the nanometer region which are not readily simulated by techniques based on gas ionization.

It should be of interest to check the calculations through measurements. A possible arrangement is schematically shown in Fig. 5. Two electron multiplier tubes are used for the detection of the low energy electrons but other systems could be used as well. In the arrangement illustrated in Fig. 5 a thin foil of conductive material is irradiated with gamma rays. These electrons will interact in the foil and secondary electrons will be emitted at the incident and exit side. If the foil is placed at a low negative retarding potential the secondary electrons will move towards electron multipliers in which they will be multiplied 10^4 times. The tubes are then connected to the charge sensitive preamplifier and so on. Coincident pulses can be stored via a pulse height analyser. It should be possible to place the foil between two slabs of the same material as the foil and thus avoid perturbing significantly the charged particle spectrum. It may be necessary to include some grids to collect the low energy electrons from the slabs themselves. All secondary electron emission work is critically dependent on the

nature of the surface. Consequently careful attention would have to be given to calibration techniques as well as to the condition of the surface.

SUMMARY

A potential technique to analyse related energy depositions in two adjacent tissue equivalent volumes is described. The technique is based on secondary electron emission from the two surfaces of a μm thick foil. The foil should thus simulate two nm thick slabs separated a distance equal to the thickness of the foil. Calculations of the coincident probability is the probability that any secondary electrons are emitted from the front surface when at least one secondary electron is emitted from the exit surface is presented to show the potential of the technique. An experimental arrangement is also discussed.

REFERENCES

- ALLER T. Mechanisms of lethal radiation damage to cells. In: *Proceedings of the second Symposium on Microdosimetry* (Verbinia p 5 Eur 4452 Commission of the European Communities, Luxembourg 1970).
- BINSTOCK D J M, BURLIN T F and SIMMONS J A. Energy deposition spectra for gamma rays and neutrons in twin volumes. In: *Proceedings of the fourth Symposium on Microdosimetry* (Verbinia p 855 Eur 5122 Commission of the European Communities, Luxembourg 1974).
- — — An application of energy deposition spectra in two associated target volumes to radiobiological effectiveness. In: *Proceedings of the fifth Symposium on Microdosimetry* (Verbinia p 633 Eur 5452 Commission of the European Communities, Luxembourg 1976).
- BIRKHOF R D, TURNER D E, ANDERSON V E, FROST J M and HAMMER N. The determination of LET spectra from energy proportional pulse height measurements. I. Track length distributions in cavities. *Health Phys* 18 (1970) 1.
- BRONSHTEIN I M and FRAJMAN H S. Inelastic scattering of electron emission from certain metals and semiconductors. *Sov Phys Solid State* 3 (1961) 1189.
- and SIGAL R B. Inelastic scattering of electrons and secondary electron emission in certain metals. *Sov Phys Solid State* 1 (1960) 1356.
- BURLIN T F. The characteristics of secondary electron emission and some potential applications to microdosimetry. In: *Proceedings of the fourth Symposium on Microdosimetry* (Verbinia p 35 Eur 5122 Commission of the European Communities, Luxembourg 1974).
- BINSTOCK D J M and HADLOW L M. A new apparatus for microdosimetric studies. In: *Proceedings of the third Symposium on Microdosimetry* (Verbinia p 658 Eur 4810 Commission of the European Communities, Luxembourg 1972).
- DIATOWITSKYA B I. Secondary emission of an ^{137}Cs caesium cathodes. *Dokl Akad Nauk SSSR* (1948) 641.
- FORSBERG H. An experimental approach to determine microdosimetric quantity for a nanometer thick volume. SSI Report 1978 034. National Institute of Radiation Protection, Stockholm 1978.
- and BURLIN T F. Microdosimetry. I. Use of secondary electron emission. *Acta radiol Oncol* 19 (1980) 115.
- KILLERER A M. Event simultaneity in cavities. The distortions of energy depositions in proportional counters. *Radiat Res* 48 (1971) 716.
- and ROSSI J H. RBE and the primary mechanism of radiation action. *Radiat Res* 47 (1971) 15.
- MCDONALD I R, LAMAKI A M and DHEAN C F. The attenuation and backscattering of electron beams by thin film. *J Phys D Appl Phys* 4 (1971) 1210.
- — — Electron emission from alkali halides under x-ray bombardment. *J Phys D Appl Phys* 6 (1973) 87.
- NAKHODKIN N G and ROMANOVSKY V A. Calculation of the secondary emission coefficient of KCl with layer thickness. *Izv Akad Nauk SSSR* 21 (1968) 179.
- NEARY G I. Chromosome aberrations and the threshold RBE. I. General considerations. *Int J Radiat Biol* (1965) 477.
- ROSSI H H. Role of associated absorption events in the radiation injury. In: *Biophysical aspects of radiation quality* p 161 IAEA Vienna 1968.

CHROMOSOME COUNTS OF ^{90}Sr -INDUCED OSTEOSARCOMAS IN MICE

III Variation of the chromosome counts of in vivo transplanted tumours, in vitro cultures and retransplanted cultured cells

H BERGMAN

As it is still technically difficult to obtain successful chromosome specimens directly from solid tumours an alternative method may be the use of a long term cultivation procedure. However this type technique may not reflect the true internal milieu of a tumour but instead create a completely new environment for the growing tumour cells. This includes impairment by factors related to the remote local defence mechanisms and loss of parameters due to the microcirculation and oxygen tension in the intact tumour etc. Such factors may well affect different cell types and perhaps also the chromosome pattern as a histologically well defined type of tumour may be and probably is a mixture of aetologically different subclasses each possibly with its own special chromosome abnormalities. Furthermore the question may arise whether it is only tumour cells or stroma cells or a mixture of both that grow. For this reason the primary purpose of the present investigation was to analyse the conformity in chromosome patterns between cultured cells from ^{90}Sr induced and parallel in vivo transplanted tumours. Another intention was to examine the consequence of retransplanting cultured cells to hosts with an apparently normal immunologic response. ^{90}Sr induced osteosarcomas were selected as an experimental model as these tumours had previously been examined for numerical characteristics (NILSSON 1962 1966 1969 1971 NILSSON & RÖNNBÄCK 1973) including the numerical chromosome progression of serially transplanted

tumours (Part I BERGMAN & NILSSON 1980 Part II BERGMAN 1980)

Material and Methods

Transplantation procedure. Only inbred, about 60-day old CBA mice were used. The primary osteosarcoma was obtained from a mouse which had received an injection of 29.6 kBq $^{90}\text{Sr}(\text{NO}_3)_3$ /g body weight. The tumour was localized by radiography 288 days after the injection. From this primary tumour the numerical chromosome progression of 55 serially transplanted generations was analysed and presented as transfer series A in a previous report (Part I). The present investigation started with transfer generation A8. Tumours from this generation were thus continuously used for in vivo transplantation (Fig. 1) but also for a parallel long term cultivation designated cultures 8.1 to 8.3. The cultures corresponded in time to the in vivo generations A9 to A11. Cells from culture 8.1 were then used for retransplantation to mice (transfer generation a12) and from the new outgrown tumours serial in vivo transplantation was performed (transfer series a).

Tissue culture methods. The tumour cultures were obtained by mincing pieces of tumour in Minimum Essential Medium (MEM) with Earle's salts. Tissue fragments were placed between cov-

erslips in a Leighton tube containing tissue culture medium 80 per cent MEM and 20 per cent foetal bovine serum plus 100 IU penicillin streptomycin per ml to 100 ml MEM was added 1 ml L glutamine. After incubation at 38°C for 48 hours the cells were rinsed with Hink's BSS removed from the coverslips with trypsin and transferred to culture bottles. Culture solution was added and after 3 to 5 days growing cell patches were observed. The outgrown cells were then transferred to another bottle after about one week. Altogether 8 passages were obtained. When the tissue cultures were harvested some of the cells were pretreated with Colcemid to a final concentration of 10^{-7} M. These cells used for chromosome analysis were incubated in hypotonic solution (sodium citrate 1%) and fixed in 1 part glacial acetic acid plus 3 parts ethyl alcohol. The fixation procedure was repeated three times. The remainder of the cultured cells from culture 8-1 was used for retransplantation. These cells were subjectively diluted with an isotonic sodium chloride solution. Ten mice were then injected subcutaneously with 0.2 ml cell suspension. From the new outgrown tumours (transfer generation 112) serial in vivo transplantation was performed.

Chromosome analysis The method used for preparation of chromosome specimens has been described previously (Part I). Only apparently well spread and unbroken metaphases were examined. Altogether 5 tumours per transfer generation were used. In successful preparations at least 25 cells per tumour were analysed. However in poor specimens it sometimes was necessary to use a reduced number and some transfer generations yielded no well spread metaphase. Due to the rather few chromosome counts per tumour and except for the tumours of transfer generation A8 and the cultures 8-1 to 8-3 only the percentile chromosome distribution per transfer generation is presented.

Histologic examination Sections of the tumours were fixed in Steve's solution, embedded and stained according to the van Gieson method or with haematoxylin eosin. The definition of tumour classification had been presented previously (Part I).

Results

Recording of chromosome abnormalities was limited to numerical variation and metacentric chromosomes. Thus numerous accidental chromosome aberrations were not recorded.

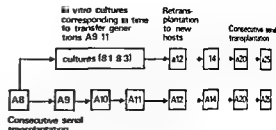


Fig. 1. Diagram of in vivo transplants (A8-A13) and in vitro cultures (8-1-8-3).

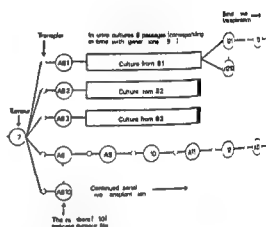


Fig. 2. Diagram illustrating in vivo transplants from transfer generations 7 to 13 (each generation consisting of 10 tumours) and the parallel in vitro cultures plus retransplantation of cultured cells.

In Fig. 1 a diagram of the transplants is presented and in Fig. 2 a diagram of the transplants but with special reference to the in vitro cultures and the retransplantation procedure. Figs 3 to 5 display cells with different chromosome numbers and different characteristics of the transfer generations and the in vitro cultures are recorded in Tables 1 and 2 respectively and the percentile chromosome distribution per generation and for the cultures in Table 3. The individual chromosome pattern of the 44 tumours (tumours 8-1-8-3) used for separate in vitro cultivation, the chromosome count of the cultures (cultures 8-1-8-3) and the variation in chromosome distribution obtained when cells from culture 8-1 were retransplanted to mice to induce new tumours (transfer generation a12) are presented in Tables 4 and 5.

Chromosome distribution The initial tumours used were derived from the 8th transfer generation. These tumours displayed a predominating number of cells within the 35 to 39 region and with peaks at 37 (43.2%) and at 35 (20.5%). The succeeding transfer generations (A9 and A10) had apart from a few



Fig 3



Fig 4



Fig 5

Fig 3 A 38-chromosome cell from a tumour of transfer generation A8

Fig 4 A 59-chromosome cell from a tumour of transfer generation A10

Fig 5 A 69-chromosome cell from a tumour of transfer generation A17

an percentile variation a similar chromosome distribution

Unfortunately no successful preparations were obtained from the 11th generation. The cell cultures 1 to 8 3 were run parallel in time with transfer generations A9 to A11. When comparing the chromosome pattern of the cultured cells with the in vivo generations a quite different distribution is revealed. Thus no cells with a lower chromosome number than 52 were found in the cultures but in

stead a predominating number of cells within the 56 to 64 region and with peaks at 60 (21.3%). When cultured cells were retransplanted to mice (transfer generation A12) tumours with a modified chromosome pattern appeared. The major changes consisted primarily of a predominating number of 40-chromosome cells (51.7%) and a more irregular distribution within the triploid region. In contrast to this generation the parallel A12 generation displayed a majority of cells within the 35 to 42 region

Table 1

Different characteristics of the in vivo generations examined

Transfer generation	No of tumours examined	No of cells counted	Mean growth period (days)	Mean size (cm ³)	Mean growth per day (cm ³)	Predominating chromosome No	Per cent	Percentage of cells with metacentrics
A8	3	132	24.3	0.73	0.030	37	43.7	9.8
A9	4	101	19.5	0.94	0.048	37	59.4	5.9
A10	3	75	20.3	1.07	0.053	37	48.0	10.0
A17	6	150	16.5	0.79	0.048	37	31.3	5.3
A14	4	95	20.5	0.88	0.043	37	48.4	0
A20	3	75	15.3	0.87	0.057	40	84.0	0
A25	3	75	14.7	0.95	0.065	40	90.7	0
A45	3	75	15.7	0.84	0.054	40	41.3	1.3
a1	4	89	22.0	1.01	0.046	40	51.7	2.2
a14	4	92	19.0	0.98	0.052	40	46.7	0
a20	7	174	16.6	1.25	0.075	60	78.7	3.3
a25	4	100	14.0	0.87	0.062	60	16.0	0

and with peaks at 37 (31.5%) and now also at 40 (24.7%). The next a generation from which successful chromosome preparations were obtained was a14. The percentage of 40-chromosome cells was now found to be 46.7 per cent and within the triploid region numerous cells within the 62 to 67 region but without distinct peaks. From generation A14 a preponderant number of 37 chromosome cells was still recorded while the 40 chromosome cells had decreased (5.3%). The next comparison was between generation A20 and a20. The A series was here characterized by a high frequency of 40-chromosome cells (84.0%) while the a series had only 10.3 per cent and within the triploid region frequently appearing cells within the 56 to 63 region and with a peak at 60 (28.7%). The final comparison was between generations A25 and a25. Concerning the A generation an even more marked dominance of 40-chromosome cells (90.7%) was recorded

while generation a25 showed distinct similarity with the preceding a generation. Sporadic analyses of the A series showed that the hitherto last examined transfer generations (A3-55) were characterized by numerous 40-chromosome cells (41.7-71.0%) but also by cells within the 52 to 67 region.

Metacentric configurations. The percentage of cells with one or more metacentrics ranged from 0 to 10 (Tables 1-2). The most metacentrics were found in the earlier transfer generations (A8-A17) of the continuously in vivo transplanted A series while for instance no metacentrics were found in the culture series (8-1-8-3).

Histologic characterization. The primary osteosarcoma A was characterized as an osteosarcoma of predominantly fibroblastic type and with a slight osteoid formation (Part I). Samples obtained from tumours of transfer generations A8 to A17 were classified as highly differentiated fibroblastic osteosarcomas with a solid growth and with pleomorphic cells. In the succeeding transfer generations the A tumours were transformed into more and more anaplastic entities while this transformation was as evident in the a series. Concerning the cell cultures (8-1-8-3) no histologic classification was performed. However of special interest was to record that the tumours of transfer generation a17 i.e. the tumours which grow out after retransplantation of cultured cells to mice were classified as highly differentiated osteosarcomas with trabecular and solid growth.

Table 2

Data of the in vitro cultures (8-1-8-3)

No of passages	8
Growth period days	60
Corresponding transfer generation	A9-11
No of tumours examined	3
No of cells counted	150
Predominating chromosome No	60
Per cent	21.3
Percentage of cells with metacentrics	0

Table 3

The percentile chromosome distribution of the transfer generations and the in vitro cultures examined

Transfer generation	No of tumours examined	No of cells counted	32	34	35	36	37	38	39	40	41	42	43	44	45	48	50	51	52
3	132		0.8	1.5	20.5	8.3	43.7	7.6	6.8	0.8	0.8						0.8		
4	101				2.0	12.9	59.4	11.9	7.0	3.9					1.0				
3	75			7.7		8.0	48.0	13.3	1.3	17.3	1.3		1.3						
6	150			0.7	2.0	6.7	31.3	7.3	1.3	24.7	6.0	2.0	0.7						
4	95				4.2	10.5	48.4	9.5	1.1	5.3									
3	75						1.3	2.7	5.3	84.0	6.7								
3	75							1.3	2.7	90.7	7.7								
3	75									41.3			1.3						
3	150																		1.3
4	101								2.2	51.7						1.1		1.1	
4	97								7.2	46.7	2	3.3		7.7					
7	174								0.6	10.3									
4	100									13.0									
Transfer generation	No of tumours examined	No of cells counted	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
3	137																		
4	101												1.0						
3	75																		
6	150																		
4	95														0.7		1.3	0.7	1.3
3	75																		
3	75																		
3	75																		
3	75																		
3	150																		
4	101																		
4	9																		
7	174																		
4	100																		
Transfer generation	No of tumours examined	No of cells counted	70	71	72	73	74	75	76	77	80	86	88	90	92	94	123	136	
3	132		0.8		1.5	0.8	4.5	1.5											
4	101					3.0	2.0		1.0										
3	75						1.3		1.3		1.3								
6	150		2.0	7.0	0.7	2.0	2.7	0.7	0.7	1.3					0.7			0.7	
4	95			1.1	5.3	6.3	3.2	1.1					1.1	1.1		1.1	1.1		
3	75																		
3	75																		
3	75																		
3	150																		
4	89																		
4	92		1.1	1.1							1.1								
7	174																		
4	100																		

Cell cultures 1-8 3) corresponding in time to transfer generations A9-11

Conclusions

The main purpose was to attain an idea whether a changed selection pressure obtained by what may be designated an immunologic defenceless in vitro

environment affects the chromosome pattern in comparison with the parallelly and serially transplanted tumours examined by a direct method and to analyse the result of retransplanting cultured cells

Table 4

The chromosome distribution of the three tumours (A8 1-A8 3) used for separate *in vitro* cultivations plus the chromosome distribution of the cultures (culture 8 1-8 3)

Tumour or culture No	No. of cells counted	Chromosome number															
		32/34	35	36	37	38	39	40	41	42/51	52/55	56	57	58	59	60	61
A8 1	48	1		2	29	5	2		1	1							
Culture 8 1	100											4	3	11	11	18	3
A8	59		27	6	13	2	4	1									
Culture 8	75										2	2	5	2	1	3	4
A8 3	75			3	15	3	3										
Culture 8 3	25										1	2	3	3	3	3	1

Table 5

Variation in chromosome distribution obtained after *in vitro* cultivation (culture 8 1) and retransplantation of cultured cells (transfer generation a12) from tumour 8 1. For comparison the chromosome pattern of the *in vivo* transfer generations A17 and A45 is presented.

Tumour culture or transfer generation No	No. of cells counted	Chromosome number															
		34	35	36	37	38	39	40	41	42	43	44/49	50/53	54	55	56	57
Tumour A8 1	48	1		2	29	5	2		1								
Culture 8 1	100															4	3
Generation a12	89						2	46				1	2	3	5	3	4
Generation A17	150	1	3	10	47	11	2	37	9	3	1						
Generation A45	75							31	1					1	2		4

to animals with a normal immunologic response. The main findings can be summarized as follows:

(1) From a ^{90}Sr induced osteosarcoma a transfer generation A8 was established by serial *in vivo* transplantation. It was found that the tumours of transfer generations A1 to A5 were characterized by a predominating number of 38 chromosome cells while the succeeding generations A6 to A8 displayed a majority of 37 chromosome cells. When tumours from transfer generation A8 were used for continued serial *in vivo* transplantation and a parallel tissue cultivation procedure different chromosome patterns were obtained.

(2) The cultures 8 1 to 8 3 varied widely (from 53 to 73) but no cells were found within the diploid region.

(3) The tumours of transfer generations A9 to A11 which corresponded in time to the culture displayed a majority of cells within the diploid region and with peaks at 37. A small number of cells was also found within the 72 to 76 region.

(4) When retransplanting cultured cells to untreated hosts (transfer generation a12) the newly grown tumours displayed a modified chromosome pattern. Thus in comparison with the cultured cell pattern a predominance of 40-chromosome cells in combination with a wide distribution of cells within the 53 to 67 region. Of special interest is to note that while no 40-chromosome cells were found in the analysis of the cultures, no less than 61.7 per cent was now recorded. In this context it should be

Table 4 (cont.)

66	67	68/71	72	73	74	75/77
				1	5	1
3				1		
		1	1		1	1
	1					
			1			
1						
1	1		2			1

Table 5 (cont.)

6	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78/99	>100
											1	5	1				
6	15	2	5	1	3						1						
1	1		3	5	4	4											1
				1		2	1	2	3	3	1	3	4	1	1	2	1
8	3																

be mentioned that in this generation no 60-chromosome cells were found in contrast to the cultures which displayed 21.3 per cent

(5) Data obtained from the *in vivo* transplanted tumours of transfer generation A12 showed a sustained predominance of 37 chromosome cells but also an increased number of 40-chromosome cells. Furthermore a few cells were found within the 67 to 77 region.

(6) Concerning the succeeding generations the split chromosome distribution was maintained even if an often marked percentile variation was observed.

(7) The A series showed by transfer generation 20 a shift of predominating chromosome number from 38 to 40 and a narrow range of variation. It should

also be pointed out that sporadic analysis of tumours from later transfer generations (A43-A55) presented a split chromosome distribution but with similarities to the pattern found in the tumours of transfer generations a12 to a25.

The results of the *in vitro* experiment indicate that an artificial environment may drastically affect the chromosome pattern. However it seems reasonable to assume that tumour cells were stimulated to grow. This opinion is supported not only by the heterogeneous chromosome pattern observed after the cultivation procedure but also by the fact that new tumours of the same histologic type could develop after retransplantation of cultured cells. It was also noticeable that these tumours immediately displayed a chromosome pattern somewhat typical of

^{90}Sr induced transplanted tumours i.e. 40 chromosome cells appearing at different stages of the tumour progression in combination with cells with in the triploid region (Part I). This chromosome distribution was also found in the serially *in vivo* transplanted tumours in transfer generations A43 to A55.

SUMMARY

This investigation started from serially *in vivo* transplanted tumours. The chromosome patterns of *in vitro* cultures and of parallelly and serially transplanted tumours were examined. Cultured cells were also used for retransplantation to mice. When comparing the chromosome counts of the cultured cells and the *in vivo* transplanted tumours, significant differences were revealed. When retransplanting cultured cells, it was also noticed that a similar chromosome distribution appeared as previously found in ^{90}Sr induced transplanted series.

ACKNOWLEDGEMENTS

The author is greatly indebted to Professor Agnar Nilsson and Docent Gunnar Walinder, the former and the present director of the Division of Radiation Biology at the National Defence Research Institute for stimulating interest and valuable advice. He also wishes to thank Pro-

fessor Jan Lindsten, Department of Clinical Genetics, Karolinska Sjukhuset for placing the facilities of his Department at the author's disposal and Miss Kersti Hansson and Mrs Siw Siljerud for their skilful technical assistance.

REFERENCES

- BERGMAN H - Chromosome counts of ^{90}Sr induced osteosarcomas in mice. II. Variation of the chromosome counts of slow and fast growing tumours in hyper and nonhyperimmunized hosts. *Acta radiol Oncology* 19 (1980) 153
- and NILSSON A - Chromosome counts of ^{90}Sr induced osteosarcomas in mice. I. Transplanted tumour series. *Acta radiol Oncology* 19 (1980) 17
- NILSSON A - Histogenesis of ^{90}Sr induced osteosarcomas. *Acta vet scand* 3 (1962) 185
- Early development of transplanted ^{90}Sr induced osteosarcoma buds. *Acta radiol Ther Phys Biol* 12 (1966) 7
- Dose dependent carcinogenic effect of radiostrontium. In: Proceedings of a symposium on radiation induced cancer organized by the International Atomic Energy Agency p. 173 IAEA STJ/PUB/278 Vienna 1979
- Pathologic effects of different doses of radiostrontium in mice. Development and incidence of leukemia. *Acta radiol Ther Phys Biol* 10 (1971) 115
- and RÖYNNÄCK C - Influence of oestrogenic hormones on carcinogenesis and toxicity of radiostrontium. *Acta radiol Ther Phys Biol* 12 (1973) 709

FROM THE SWEDISH RESEARCH INSTITUTE OF NATIONAL DEFENCE DEPARTMENT 4 S 104 90 STOCKHOLM
 AND THE DEPARTMENT OF PATHOLOGY FACULTY OF VETERINARY MEDICINE SWEDISH UNIVERSITY OF
 AGRICULTURAL SCIENCES S 750 07 UPPSALA SWEDEN

AGE AND DOSE RELATED CARCINOGENICITY OF ^{90}Sr

A NILSSON P BIERKE G WALINDER and A BROOMÉ KARLSSON

It seems to be increasingly important to elucidate various biologic parameters such as hormonal metabolic imbalances genetic constitution sex and age may influence upon or modify the effect of radiation

As regards age there are quite definite changes in the susceptibility to the induction of various types of tumours as a function of the postnatal age at which animals are irradiated (UNSCEAR 1977). It is also known (DECKER et al. 1964 ANDERSON & COMAR 1968) that ^{90}Sr is retained maximally when administered to dogs of an age of 5 to 12 months, i.e. somewhat before puberty, whereas when given earlier the retention is reduced largely on account of a high requirement for bone resorption to allow for growth. At a later age a much reduced resorption and accretion was observed. Experiments performed by PECKMAN & NORRIS (1964) with mice and rats also clearly pointed in the same direction. Young animals (1-30 days of age) retained less strontium than young adults (42-101 days of age) whereas old animals retained the lowest amount. Numerous reports have stated a relationship between the body burden of ^{90}Sr and frequency of tumour bearing animals as well as multiplicity of tumours which indicates a strongly age related hazard reaching a maximum just before or during puberty. To which extent this age related susceptibility is also associated with a specific sensitivity of the tissues at different ages or only reflects an increased body burden is not well understood. Reports by VAN

PUTTEN (1969) and FRANKEL & MILLER (1964) indicate that the tissue sensitivity to cancerogenic effects of internal emitters under the conditions prevailing in their experiments does not significantly change with the age of the animal.

The main purpose of the present report is to present the mutual connection between injected amount of ^{90}Sr and the age of mice upon factors such as survival frequency of osteosarcomas and lymphoreticular tumours (LR tumours) as well as the incidence of soft tissue tumours.

Material and Methods

Three different doses of ^{90}Sr —given intraperitoneally as $^{90}\text{Sr}(\text{NO}_3)_2$ —were supplied to 3 series of inbred CBA female mice subdivided into 4 different age groups (Table 1).

The mice were during the whole experimental period kept in the same room and inspected twice daily. They were grouped at random and maintained in cages with 10 animals in each. A commercial pelleted diet (Standard Feed for Rats and Mice, Astra Ewos) and water were supplied ad libitum.

Autopsy was carried out when the animals were moribund or in a near moribund state. Before autopsy radiography of each mouse was performed in order to detect bone tumours. The carcasses were routinely weighed as well as the liver, spleen

Table 1
Experimental design

No of mice	Injection on day	Inj $^{90}\text{Sr/g b w}$
		kBq (μCi)
50	300	29.6 (0.8)
50	150	
50	75	
50	25	
50	300	14.8 (0.4)
50	150	
50	75	
50	25	
50	300	7.4 (0.2)
50	150	
50	75	
51	25	

The asterisks indicate that during the course of the experiment (*) one (**) and (***) 3 animals were lost

thymus and brachial and axillary lymph nodes. These organs, all macroscopic hard and soft tissue tumours and radiographically suggested bone tumours were fixed in Steeve's fluid. Hard tissues were decalcified in 20 per cent formic acid. Ordinary histologic techniques were used and the sections were routinely stained according to the van Gieson method and with haematoxylin-eosin.

The diagnosis of bone tumours was based upon radiographic post mortem and microscopic findings. The number of intraosseous bone tumours recorded is a minimum since only bones with radiologically suggested tumours were examined microscopically. All tumours were classified according to the nomenclature preferred by the EULEP (European Late Effects Project Group) Committee on Pathology Standardization.

Results

Survival time The age of the animals within the different dose groups had a very small influence on their remaining survival times (Table 2). Thus, in the 29.6 kBq group all animals endured approximately 300 days. In the 14.8 kBq group the corresponding value was about 400 days except for the mice injected on day 25 which lived significantly shorter than the other age series in the same group ($p < 0.01$).

In the lowest dose group a significant negative correlation ($r = -0.997$) was observed between survival times and ages.

The bone tumour induction time was roughly the same irrespective of the age at the time of ^{90}Sr administration within both the 29.6 and the 14.8 kBq group. In the lowest dose group a probable negative correlation ($r = -0.97$, $p < 0.05$) between induction times and ages was found.

Bone tumour incidence The number of tumours bearing mice as well as the total number of tumours are recorded in Table 2. If the osteosarcoma incidence is expressed as the number of tumours per mouse or better the mean tumour induction rate in 50 mice (MTIR, Table 2) it is discernible that the age of the animals at the time of ^{90}Sr administration has a great influence on the future appearance of bone tumours. The MTIR is defined as the regression coefficient of the tumour frequency in 50 mice as a function of time between T-SD and T+SD where T is the latency time between injection and the clinical appearance of the tumour and SD is standard deviation. The MTIR data reveal a highly statistically significant difference of tumours between all age groups irrespective of the dose of ^{90}Sr .

Based upon the MTIR data (Table 2) it is evident that the adult 75 day old mice are always more prone to develop tumours. Then follow the mice injected on day 25 and on the third and fourth place those given ^{90}Sr on day 150 and 300 respectively. It should also be noted that mice given 14.8 kBq/g b.w. on day 75 belong to the same sensitivity level as mice given 29.6 kBq/g b.w. on day 300. The same can be observed in mice given 14.8 kBq/g b.w. on day 150 and those injected with 7.4 kBq/g b.w. on day 25 and 75. However, the mean induction times for the tumours as well as the total number of tumours differ substantially between the different dose groups. As could be expected no correlation exists between survival times and total number of tumours even in cases where the MTIR figures are similar.

Lymphoreticular tumour incidence The frequency of LR tumours is unrelated to the age of the animals when injected with the nuclide (Table 1). However, the two lowest dose levels are much more tumorigenic than the highest one.

Other neoplasias Very few soft tissue tumours were observed at the highest dose level irrespective of the age at injection of ^{90}Sr (Table 4). The maximum of soft tissue tumours (70) were found at the lowest

Table 2

The incidence of ⁹⁰Sr induced bone tumours related to age and dose and expressed by the mean tumour induction rate

⁹⁰ Sr/g b w (μ Ci)	Inject on day	No of mice	Mean survival days \pm SE	Total No of tumours	No of mice with tumour(s)	Mean induction time (T) days \pm SE	No of tumours per mouse	MTIR	MTIR level *
5 (0.8)	300	50	277 \pm 9	80	40	793 \pm 5	1.60	0.641 \pm 0.032	4
	150	47	312 \pm 10	113	47	370 \pm 8	1.98	0.840 \pm 0.022	3
	75	48	795 \pm 6	149	47	301 \pm 7	3.10	1.662 \pm 0.047	1
	75	50	797 \pm 7	84	40	319 \pm 3	1.68	1.105 \pm 0.055	2
8 (0.4)	300	50	413 \pm 17	63	35	449 \pm 11	1.26	0.332 \pm 0.013	6
	150	49	406 \pm 18	46	32	490 \pm 20	0.94	0.182 \pm 0.012	7
	75	49	397 \pm 14	113	40	434 \pm 6	2.31	0.624 \pm 0.077	4
	25	49	373 \pm 24	69	28	340 \pm 7	1.41	0.519 \pm 0.014	5
4 (0.2)	300	49	516 \pm 21	15	14	572 \pm 38	0.31	0.0372 \pm 0.0046	9
	150	47	569 \pm 27	14	14	667 \pm 28	0.30	0.0595 \pm 0.005	8
	75	49	600 \pm 23	57	30	683 \pm 14	1.16	0.213 \pm 0.0059	7
	75	51	675 \pm 30	33	24	710 \pm 15	0.65	0.133 \pm 0.019	7

The mean tumour induction rate (MTIR) is defined as the regression coefficient of the tumour frequency in 50 mice as a function of time between T-SD and T+SD where T is the latency time between injection and the clinical appearance of the tumour and SD its standard deviation

*The level of MTIR given by numbers indicates a highly significant difference ($p < 0.001$) in MTIR between successive numbers

Table 3

Incidence of lymphoreticular (LR) tumours in relation to age and injected dose of ⁹⁰Sr

Age group (days)	⁹⁰ Sr dose kBq (μCi)/g b w						Total (per cent)
	29.6 (0.8)		14.8 (0.4)		7.4 (0.2)		
	No of mice	No of mice with LR tumours	No of mice	No of mice with LR tumours	No of mice	No of mice with LR tumours	
300	50	0	50	7	49	0	13 (8.7)
150	47	2	49	6	48	10	18 (12.5)
75	48	1	49	4	49	9	14 (9.6)
25	50	1	49	8	51	2	11 (7.4)
Total (per cent)	195	4 (2.1)	197	25 (12.7)	197	27 (13.8)	

Of these 41 per cent were confined to the group of animals injected on day 300. If the whole material is taken into consideration the predominance of this group will still be 41 per cent. With a few exceptions the main part of the tumours were restricted to the liver, uterus, ovary, lung and mammary gland. In the lowest dose group the spectrum of different tumours was more widespread. In the 14.8 kBq group a slight tendency to a higher tumour frequency in the two oldest age groups was

found (13 and 9 in the groups injected on day 300 and 150 respectively against 5 and 5 in the two youngest).

The majority of the liver tumours were hepatocellular adenomas (hepatomas). In one case a hepatocellular carcinoma (malignant hepatoma) with metastasis to the lung was found. The uterine tumours were either leiomyoma or leiomyosarcoma. All ovarian neoplasias were granulosa cell tumours. The pulmonary tumours were all classified as al

Table 4

Soft tissue tumours survival time and lifespan in groups of mice injected with different doses of ^{90}Sr at 300 150 75 and 25 days of age

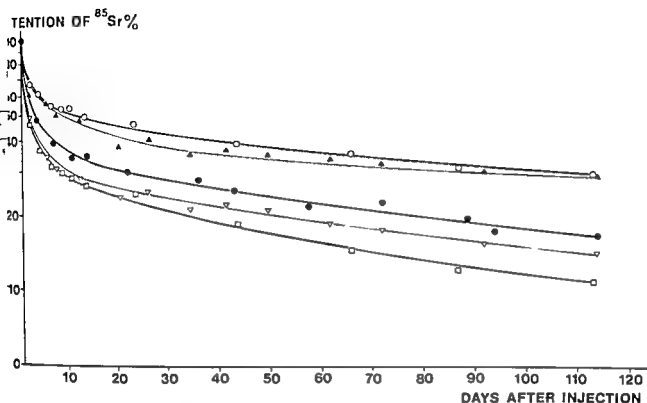
Organ with tumour	^{90}Sr dose kBq (μCi)/g b w											
	29.6 (0.8)				14.8 (0.4)				7.4 (0.2)			
	Injected on day				Injected on day				Injected on day			
	300	150	75	25	300	150	75	25	300	150	75	25
Liver	-	1	-	-	5*	4	1	1	10	5	3	7
Uterus	-	1	-	-	2	2*	2	1	5**	-	2	1
Ovary granulosa cell tumour	-	-	-	-	3	-	-	-	2	-	1	-
Lung alveolar cell carcinoma	-	-	-	-	2	2	-	1	6	2	3	7
Mammary gland	-	-	-	-	1	1	1	1	-	-	1	-
Skin and subcut tissue	1	-	-	-	-	-	-	-	-	4	-	-
Harderian gland	-	-	-	-	-	-	-	1	1	-	-	-
Adrenal gland	-	-	-	-	-	-	-	-	2	-	-	-
Phaeochromocytoma	-	-	-	-	-	-	-	-	-	-	-	-
Spleen haemangioma	-	-	-	-	-	-	-	-	-	-	2	-
Pituitary adenoma	-	-	-	-	-	-	1	-	-	-	-	-
Unclassified	-	-	-	-	-	-	-	-	3	-	2	1
Total No. of tumours	1	2	0	0	13	9	5	5	29	11	11	16
No. of mice	50	47	48	50	50	49	49	49	49	47	49	51
Survival time days M \pm SE	772 \pm 9	312 \pm 10	295 \pm 6	297 \pm 7	413 \pm 17	406 \pm 18	392 \pm 14	323 \pm 24	516 \pm 21	469 \pm 77	600 \pm 136.5	745 \pm 16.5
Lifespan days M \pm SE	572 \pm 9	467 \pm 10	370 \pm 6	322 \pm 7	713 \pm 17	556 \pm 18	467 \pm 14	348 \pm 24	816 \pm 71	719 \pm 77	675 \pm 360	640 \pm 77
One malignant hepatoma	Schwannoma (2) Seb. gland adenoma (2)											
Leiomyosarcoma	Adenomas and adenocarcinomas											
Four leiomyosarcomas	one leiomyoma											

Table 5

Probable causes of death in groups of mice injected with different doses of ^{90}Sr at 300 150 75 and 25 days of age

Inj ^{90}Sr /g b w	Injected on day	No. of mice	Osteo sarcoma	LR tumours	Other tumours	Inantion	Neg autopsy	Other causes
kBq (μCi)								
29.6 (0.8)	300	50	34	0	1	10	5	0
	150	47	36	2	1	5	3	0
	75	48	45	1	0	0	2	0
	25	50	33	1	1	14	0	2
14.8 (0.4)	300	50	25	7	6	6	4	2
	150	49	26	6	5	4	6	1
	75	49	37	4	0	1	6	0
	25	49	23	8	4	10	4	1
7.4 (0.2)	300	49	4	6	21	8	9	1
	150	48	8	10	11	12	9	2
	75	49	23	9	8	3	4	0
	25	51	17	2	9	14	9	0

Emaciation including general hypoplasia of lymphoid and blood forming tissue



Retention of ^{85}Sr in CBA male mice of different ages (\square 168 days, \bullet 75 days, \blacktriangle 50 days, \circ 30 days) at the time of

exposure in relation to time. Each point represents the mean value of 10 animals (After Ronnback)

olar cell carcinomas. Those of the mammary glands were either adenomas or adenocarcinomas. No subcutaneous tumours of the chest were observed. Both tumours of the adrenal glands were pheochromocytomas. Six tumours were not possible to classify on account of autolysis.

Causes of death. The most probable causes of death appear in Table 5. In the two highest dose groups the majority of animals, irrespective of age at the time of ^{85}Sr injection, have died from osteosarcomas or LR tumours. In the lowest dose group an increased number of animals died of other causes such as soft tissue tumours and inanition, particularly in the series injected on day 300. In a few cases also inflammation was found. In several animals the cause of death could not be ascertained.

Discussion

The MTIR in groups of 50 mice was considered to be more independent of survival times and much more sensitive and reliable in expressing the osteosarcoma incidence than merely using the number of tumour-bearing mice, particularly since it is a well-known fact that the multiplicity of ^{85}Sr induced tumours is dose related (NILSSON, 1970). When com-

paring the various age series at the three different dose levels, the existence of a strong age related sensitivity was evident from the fact that the young adult mice (75 days old) always were more inclined to develop bone tumours than were young mice (25 days old) or mid aged (150 days old) and old mice (300 days old). The reason for this constant finding may above all be associated with the age-determined retention of radiostrontium as has been evidenced by RONNBACK (1979) using ^{85}Sr (Figure) and by among others DECKER et al., COLL, ANDERSON & COMAR and SPECKMAN & NORRIS.

Since no estimations of the radiation dose have been made for the tumour induction in the different age groups, it is not possible to express any opinion about variation of the specific sensitivity with age of the bone tissue to the cancerogenic effect of ^{85}Sr .

The difference of survival time observed between young and young adults on one hand and the old animal group on the other seems largely to be related to the high initial age of the latter when inserted in the experiments. In fact the age of these animals ($516 \pm 300 = 816$ days) is fairly high as compared with the mean life span of female CBA mice (823 ± 22 7 days, NILSSON & BROMÉ-KARLSSON, 1976). The reason for the short survival time of the young mice

in the 14.8 kBq group is difficult to explain but may partly be related to the increased incidence of LR tumours and enhanced rate of maturation including hypoplasia of lymphoid and blood forming tissues partly it may also be associated with the fact that animals which are still growing are more vulnerable to this ^{90}Sr dose (14.8 kBq/g b.w.) than mice in the other age groups. This explanation may have some support by the fact that the induction time for the bone tumours is also significantly reduced among the young animals ($t=4.263$, $p<0.001$) as compared with the remaining mouse groups in the 14.8 kBq series. In the highest dose series this difference will not be recognized because in these series the irradiation dose seems to be equally dangerous regardless of age.

No relationship was found between the incidence of LR tumours and age which is in a sharp contrast to the experience when external irradiation is used (KAPLAN & BROWN 1952; JARPLID 1968). The reason for this seems to emphasize previous observations (NILSSON 1971; JARPLID 1974) that ^{90}Sr initiates LR tumours within the bone marrow by a mechanism which differs fundamentally from that in the thymus after fractionated external irradiation.

In the present experiments soft tissue tumours were significantly less frequent in the high than in the low level dose series. Thus of a total of 105 tumours 67 per cent were found in the low and 30 per cent in the mid level dose. Taking into account that the animals in the low level series have been exposed to irradiation for a longer time than in the other series the age seems to be a more decisive factor for tumour development than the dose. The mouse groups exposed on day 300 have approximately double the amount of tumours as compared with the groups injected on day 75 and 25. This evaluation also seems to have support from the fact that most tumours occurred in the group with the longest total life span (816 days) i.e. in the group injected with 7.4 kBq/g b.w. on day 300 in spite of being exposed to ^{90}Sr significantly shorter (516 ± 21 days) than the group injected on day 25 (625 ± 30 days) at the same dose level.

SUMMARY

^{90}Sr was given at three different dose levels (29, 14.8 and 7.4 kBq/g b.w.) to groups of mice aged 300, 150, 75 and 25 days. It was found that the incidence of osteosarcomas was highest in the 75-day-group and lowest in the two oldest age groups. The frequency of lymphoreticular

tumours was inversely dose related (highest incidence in the lower dose series) and not dependent on age at the time of ^{90}Sr injection. The frequencies of soft tissue tumours indicate that these tumours are more related to dose than to the dose employed.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Dr Curt Ronnback for his generous contribution with the ^{90}Sr retention curve. This investigation was supported by grants from the Swedish Cancer Society (Project No. 790 B77 03XC) and was also carried out as part of the program of the European Late Effects Project Group (EULEP).

REFERENCES

- ANDERSON J. J. II and COMAR C. L. Strontium retention as a function of age in the dog. *Radiat. Res.* 34 (1967) 153.
- DECKER C. F., KASPAR L. V. and NORRIS W. P. The variation of strontium metabolism with age in the dog. *Radiat. Res.* 23 (1964) 475.
- FINNELA J. and MILLER C. E. Radium retention in mice as a function of age. *Radiat. Res.* 22 (1964) 188.
- JARPLID H. Radiation induced asymmetry and lymphoma of thymus in mice. *Acta radiol.* (1968) Suppl. No. 179.
- Combined effect of roentgen irradiation and ^{90}Sr on the haematopoietic tissues and the development of lymphoma in mice. *Acta radiol. Ther. Phys. Biol.* 13 (1974) 217.
- KAPLAN H. S. and BROWN M. B. Protection against radiation induced lymphoma development by shielded and partial body irradiation of mice. *Cancer Res.* 13 (1952) 44.
- NILSSON A. Pathologic effects of different doses of radiostrontium in mice. Dose effect relationship of ^{90}Sr induced bone tumours. *Acta radiol. Ther. Phys. Biol.* 9 (1970) 155.
- Pathologic effects of different doses of radiostrontium in mice. Development and incidence of leukaemia. *Acta radiol. Ther. Phys. Biol.* 10 (1971) 115.
- BROOME KARLSSON A. Influence of steroid hormones on the carcinogenicity of ^{90}Sr . *Acta radiol. Ther. Phys. Biol.* 15 (1976) 417.
- VAN PUTTEN L. M. The uptake and toxicity of ^{90}Sr in mice fed to mice at different ages. *Int. J. Radiat. Res.* 13 (1967) 380.
- Influence of mouse age on bone tumour frequency from ingested strontium 90. IAEA SM 118/19 p. 37. Vienna 1969.
- RÖNNBACK C. Unpublished observation (1970).
- UNSCEAR. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and effects of ionizing radiation. Report to the General Assembly 1962. New York 1977.
- SPECKMAN T. W. and NORRIS W. H. The age-dependence of strontium retention in rats and mice. *Radiat. Res.* 22 (1964) 461.

DOSE DISTRIBUTION AROUND RADIUM ARRAYS USED IN THE TREATMENT OF UTERINE CARCINOMA

P. D. SHARMA and J. C. F. MACDONALD

The determination of absorbed dose in gynecologic applications of brachytherapy sources has always been more difficult and less accurate than in any other branch of radiation therapy. This difficulty is inherent in a technique which involves using linear sources at short distances where the inverse square law gives rise to large dose gradients. Despite the advent of the electronic computer and the consequent improvement in the accuracy of calculated dose distributions, still many uncertainties are involved, not the least the positions and shapes of relevant organs.

HEYMAN (HEYMAN et coll. 1941) introduced the so-called packing technique in which the uterus is packed to capacity with a number of spacing filters enclosing radium tubes. When the technique is properly executed the uterine wall is fixed relative to the radium so that when the exact location of the tubes is determined it is possible to deduce an isodose distribution applicable to the size and shape of the uterus in question. Stereoscopic films are helpful in the localization of the tubes but they are only the first link in a long chain of tedious procedures that must be performed before an accurate dose distribution can be produced.

BENNER (HEYMAN et coll. 1941; HEYMAN & BENNER 1946) considered that the resultant dose distribution should not be critically dependent upon the positions of the radium sources and for this reason he placed little emphasis on exact localization procedures. On this assumption he produced a

set of tables linking the number of sources to an optimum treatment time which was based on an estimation of resultant dose rates at 1.5 cm from the uterine wall. From a modern dosimetric viewpoint this approach is unsatisfactory since the method used for treatment time calculation involved no correction for self absorption among the tubes themselves nor any allowance for the shape of the array. In addition the type of filter originally used by HEYMAN bears little resemblance to that commonly in use today. A comparison of two Heyman applicator filters with a modern Campbell type stainless steel filter is made in Fig. 1. It is seen that these are considerably different in physical dimension and so one would expect the result in dosimetry to be considerably different.

In his measurements BENNER used ionization chambers to determine the dose at short distances from the array. Because of the large size of these detectors the spatial resolution was poor. All measurements were made on the surface of a simulated uterus in air and doses were only recorded at a fixed distance from the radium which was assumed to correspond to the outside wall of the uterus.

In the present work the following problems were analysed: (1) what the uterus receives during a typical insertion of present-day applicators and (2) how valid the tables originally deduced by BENNER are when applied to existing techniques.

Table 1

Comparison of experimental and theoretic values for a single 10 mg radium tube filtered through 1 mm of platinum Source diameter 0.29 mm active length 1.25 cm

Experimental points as defined by YOUNG & BATHO		Calculated values from YOUNG & BATHO (mGy/h)	Experiment (mGy/h)
X	Y		
0	1	649	629
0	2	176	169
0	3	79	73
0	4	44	47
0	5	28	32
0	6	19	21

X is the displacement of the perpendicular from the center of the active length of the source

Y is the perpendicular distance of the point from the axis of the source

In examining these problems measurements were made using teflon coated 1 mm × 6 mm lithium fluoride thermoluminescent microrod dosimeters these were employed because of their small size and good spatial resolution. The measured response of these detectors is a function of their own structure and of the readout device. It is therefore necessary to perform an absolute calibration at the time of each measurement to eliminate any short term variation in the sensitivity of the system.

Calibration

Thermoluminescent LiF microrods manufactured by the Harshaw Chemical Company were used for the dosimetric determinations.

The energy dependence of these dosimeters in the photon energy range up to 1.25 MeV is shown in Fig 2. It appears that between $E = 137$ keV (3 mm of Cu HVT) and 1.25 MeV (15 mm of Cu HVT) the maximum variation in response is 5 per cent. At lower energies the energy dependence is significant with an enhanced response of up to 40 per cent relative to the response at 1.25 MeV.

The overall reproducibility of measurement using these detectors was found to be adequate for the present purposes. Sets of 20 measurements at each of a number of dose levels produced a standard deviation of 5 per cent. In the first 48 hours after irradiation a drop of about 10 per cent in response occurred after which the dosimeter showed no fur-

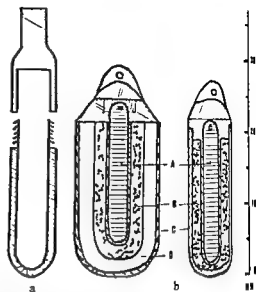


Fig 1 Comparison of a) Campbell type applicator and b) Heyman irradiators. A Radium tube B Inner wall of lead C External wall of stainless steel D Intermediate space filled with air

ther significant fading with time. Accordingly the microrods were calibrated by irradiation to a known dose of ^{60}Co radiation and the practice of reading the dosimeters two days after irradiation was instituted. The justification for using these dosimeters is that in sharp dose gradients the positional accuracy is a limiting factor. For example at 2.0 cm from radium tubes the dose rate varies by about 10 per cent per mm. An estimate of the reproducibility of this system is ± 1.0 mm so that an overall accuracy of ± 15 per cent is all that can be expected.

The experimental method was first checked by evaluating the dose distribution in water from a single 10 mg radium tube. A polystyrene plate immersed in a water phantom held the radium source

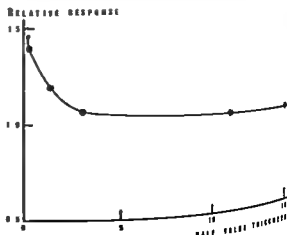


Fig 2 Energy dependence of LiF microrods

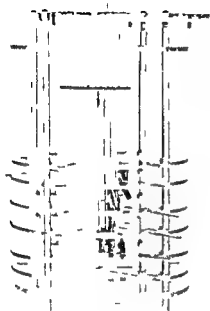


Fig 3 Source and detector jig



Fig 4 a



Fig 4 b

Fig 4 a) A p film of the actual packing b) Control film of the source support

entered in a polar array of lithium fluoride microrods. The measured dose rates at various distances were compared with the values calculated from BATHO tables (BATHO & YOUNG 1964, YOUNG & BATHO 1964). The results are compared in table 1. It is seen that at distances greater than 4 m the measured values are high by an amount greater than the experimental uncertainty. The contribution of low energy scattered radiation at these distances together with the increased sensitivity of the dosimeter for low energies could give rise to this increased response. Lithium fluoride microrods were thus found suitable for measurements under water.

Apparatus

A 45 cm × 45 cm × 40 cm deep tank made of clear plastic and filled with water was used for the measurements. The source and detector supports (Fig 3) were made of water equivalent plastic and were

rigidly fixed in relation to each other so that any arrangement could be accurately reproduced. The detector support consisted of six plastic rings mounted on four uprights fitted into a shallow recess in the bottom of the tank. These uprights were graduated so that the vertical position of the rings could be recorded and reproduced. Each ring carried six polystyrene strips spaced around it at intervals of 60 degrees. The lithium fluoride rods were fitted into slots in the strips. Each strip could move in the slot and was graduated so that its radial position could be reproduced.

In order to hold the radium sources in position a support (Fig 3) fitting tightly into the top of the detector jig was constructed. The support was made up of two spaced plastic plates into which a matrix of holes was drilled. Polystyrene rods bent to any desired shape by heat treatment were then pushed through the appropriate holes and clamped in position. Thin walled polystyrene holders were attached to the ends of these rods. In this way it was possible

to reproduce virtually any clinical radium distribution the presence of the polystyrene rods supporting the lower sources would occasionally interfere with the exact orientation of the upper sources

Experimental procedures

A p and lateral films of eight typical Heyman treatments form the basis of the reconstructed radium arrays. The polystyrene support rods were adjusted to produce as nearly as possible a model of the actual packing arrangement. The accuracy of this procedure was controlled by exposing films of the source support with the dummy sources in place (Fig 4)

Microrod dosimeters were distributed on the detector strips the strips were mounted on the rings and then pushed into their final positions. Campbell applicators (a modern version of the Heyman filters) each containing a 10 mg radium tube were then placed in the holders in the source support which was then inserted in the detector array. Thereafter this entire arrangement was immersed in the water tank and left for an irradiation time of 20 to 24 hours the time depending upon the number of sources used. During this period films of the arrangement were exposed in two directions at right angles the geometry being chosen to make the magnification correspond to that of the original films of the patient. With the radium removed the dosimeters were read together with the calibration detectors using a Tele-dyne TLD 7300 reader. From a graph of dose rate against position along each strip the dose rate distribution at the level of each ring was determined. From the distributions in the six horizontal planes dose rate distributions in the other two mutually perpendicular planes could be produced by interpolation. By using the known magnification factors it was possible to transfer the known dose rate contours directly onto the original films.

The computation of the dose distributions was performed using an Artronix PC 12 computer and employing as input the coordinates of the ends of the applicators as determined from measurements on pairs of orthogonal films. This straight forward calculation was complicated by problems of cross filtration frequently the dose at a point from a particular source would be reduced because of absorption by one or more other source capsules. In addition the extra filtration provided by the spacing filters had to be taken into account in the calculations. The

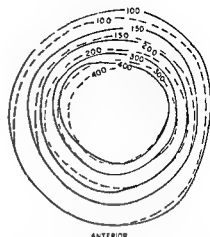


Fig 5 Dose distribution (in mGy/h) for the 4 tube array. () measurement (—) Computer calculation (---)

contribution of these factors was measured experimentally using thermoluminescent dosimeters. For two Heyman encapsulated radium tubes the cross filtration effect was determined to be 3 per cent at a 2.5 cm distance and the effective absorption from the filters was 4 per cent. In order not to complicate the computer calculations a constant absorption factor of 8 per cent for each filter was employed.

The results of the computer calculations and the experimental measurements for a particular array in a specific plane appear in Fig 5. In this case it may be concluded that the agreement is within the region of the combined error of the two methods. A total of 8 arrays ranging in activity from 5 to 16 mg radium tubes were analysed. In each case a comparison of the results of dose measurements with those of computer calculations were compared with values derived from BENNER's tables. The comparison at a distance of 15 mm from the external surface of the radium a distance which is consi-

Table 2
Experimental measurements of Heyman arrays

No	Number of Ra tubes	Measurements at 15 cm (mGy/h)	Calculated from BENNER's tables (mGy/h)	Comparison (mGy/h)
1	5	394 ± 60	458	411
2	5	576 ± 80	458	463
3	6	459 ± 70	500	413
4	6	356 ± 50	500	361
5	8	468 ± 70	565	464
6	8	493 ± 70	565	464
7	12	696 ± 100	700	614
8	16	880 ± 170	804	814

red to be equal to the average thickness of the uterine wall is shown in Table 2

Accuracy In the radiation measurements with a reproducibility of source and detector of approximately ± 1.0 mm an overall dose determination accuracy of ± 15 per cent is expected

In the computer calculations the accuracy of the computer dose rate depends upon the accuracy of determination of the end points of the sources from measurements on the films This again is estimated to be ± 10 per cent Other sources of uncertainties lie in the assumption of the effects of cross filtration and in the variation in the true activity content of the tubes The overall effect of these variables leads to an expected accuracy of ± 15 per cent

Conclusions

The HEYMAN packing method from its inception has been recognized as a system that is not amenable to accurate dosimetry In his pioneer work BENNER recognized this and on the basis of experiments that would be considered crude in today's standards recommended a method to form a routine basis for dose evaluation

Computers at present available make possible the determination of dose at any point in the vicinity of a radioactive array the principal problem is in many cases the specification of significant points at which the dose should be determined In the packing technique BENNER's points of interest 15 mm beyond the outer most radium may still be considered to be a reasonable region of interest

In the present work measurements have been made to determine the dose distribution around eight typical packing method arrays of commercially available Heyman applicators A comparison of the results of these measurements with the dose rates derived from BENNER's tables shows satisfactory agreement even when applicators are employed that are considerably different from those used by HEYMAN

Further it has been established that standard dosimetric computational techniques produce re-

sults which are in agreement (within acceptable levels) with those of the results of measurement and with the values of BENNER

This work supports BENNER's approach to the Heyman technique in that a reasonably uniform dose distribution can be achieved from a haphazard array of tubes and that this dose distribution is insensitive to small changes in techniques

SUMMARY

Measurements have been made to determine the dose distribution around typical arrays of commercially available Campbell type Heyman applicators using thermoluminescent dosimeters These measurements verify the tables produced by BENNER linking the number of sources used in the so called packing technique for the treatment of uterine carcinoma to an optimum treatment time

ACKNOWLEDGEMENT

This work was supported by a research grant from The Ontario Cancer Treatment and Research Foundation Project Numbers 282 and 297 which is greatly acknowledged

REFERENCES

- BATHO H F and YOUNG M E J Tissue absorption corrections for linear radium sources *Brit J Radiol* 37 (1964) 689
- A revised table for tissue correction factors for linear radium sources *Brit J Radiol* 40 (1967) 785
- COHEN M JONES D M A and GREENE D Central axis depth dose data for use in radiotherapy *Brit J Radiol* (1972) Suppl No 11 p 107
- HEYMAN J The radiotherapeutic treatment of cancer of the corpus uteri *Brit J Radiol* 20 (1947) 85
- and BENNER S Further experience with radiotherapy in cancer of the corpus of the uterus *Acta radiol* 27 (1946) 328
- REUTERWALL O and BENNER S The Radiumhemmet experience with radiotherapy in cancer of the corpus of the uterus *Acta radiol* 22 (1941) 11
- YOUNG M E J and BATHO H F Dose tables for linear radium sources calculated by an electronic computer *Brit J Radiol* 37 (1964) 38

FROM THE LABORATORY OF EXPERIMENTAL BIOLOGY, DEPARTMENT OF ANATOMY, THE DEPARTMENT OF RADIATION THERAPY, UNIVERSITY OF GOTHENBURG, S-40033 GOTHENBURG, AND THE INSTITUTE FOR APPLIED BIOTECHNOLOGY, S-43122 MOLNDAL, SWEDEN

IRRADIATION INJURY OF BONE TISSUE

A vital microscopic method

T ALBREKTSSON, M JACOBSSON and I TURESSON

Numerous reports (for review see NATHANSSON 1977) deal with the effect of irradiation on bone tissue but many central questions remain unsolved such as for instance whether bone necrosis after irradiation is preceded by a vascular injury. Histology and microangiography after irradiation are indirect methods and provide no repeated evaluation of the events occurring in the injured bone. Only a direct method also enabling a repeated observation of the same tissue compartment at different stages after defined irradiation dosage would allow more definite conclusions concerning the effect of irradiation. The titanium bone chamber is an attempt to overcome drawbacks connected with the indirect methods. Using the bone chamber, vital microscopy of bone tissue and vessels becomes possible both before and at repeated intervals after irradiation. Direct comparison can thus be performed and the development of the tissue injury can be recorded in situ. Previous vital microscopic methods applied to the examination of soft tissues such as the rabbit ear chamber (SANDISON 1924) or the mouse skin chamber (ALGIRE 1943) have also been used on irradiated bone tissue (SANDISON 1928, KIRBY SMITH 1933, CLARK & CLARK 1942, KIEHN et coll 1952, EZRA COHN et coll 1969, SUDMANN 1975). These methods, although allowing a repeated observation of bone tissue changes in the living animal, are limited to heterotopical bone grafts. No evaluation of the behaviour of bone tissue *in situ* is possible.

However, this can be performed using the titanium bone chamber now described.

Material and Methods

Bone chamber and recording procedures. The titanium bone chamber (Fig 1a) consists of a hollow screw into which one short and one long glass rod are glued leaving a 100 micron wide space in between. If the chamber is inserted into a long bone of a rabbit using a gentle surgical technique, bone and vascular tissue will grow into this space during a healing period of 4 to 6 weeks. With the animal in light anaesthesia it is then possible to observe the ingrown tissue in the vital microscope. The bone and vascular structure are recorded on film and slides.

Vital microscope. A modified Leitz intravital microscope was used for all *in vivo* recordings. As a rule, magnifications between 20 \times and 100 \times were tried. A Vinten scientific 16 mm film camera and a Leica camera were connected to the vital microscope. Kodachrome 25 cine film and Ektachrome 200 film were used.

Animals and anaesthesia. Preliminary experiments were performed on 5 healthy adult rabbits (Belgian hare) 10 to 16 months of age, weighing 4.5 to 6.0 kg. During surgery, irradiation and vital

microscopic sessions the animals were under general anaesthesia maintained by intramuscular injections of Hypnorm (Mekos Helsingborg Sweden) at a dose of 0.7 ml/kg body weight. The Hypnorm drug is a combination of Fentanyl and Fluanison.

Irradiation procedure The rabbits were irradiated at a temperature of 22°C. During the irradiation the bone of the animal was placed upon a 10 cm thick polystyrene phantom. A ^{60}Co unit (Siemens Gammatron G3) was chosen to minimize the difference in the absorbed dose in soft tissue and bone and in the disturbances in the radiation field due to the titanium chamber. Source skin distance was 60 cm and the field size was 5 cm \times 5 cm. The dose rate was 1.20 Gy/min. A 5 mm bolus of silicone rubber material was applied to ensure full build up as the top of the bone chamber was only 2 mm below the skin surface (Fig. 2). Each rabbit was given 15.0 Gy (the absorbed dose in water at 5 mm corresponding to the position of the chamber). The appearance of the bone was recorded during the healing period following surgery and 2 weeks later. The irradiation was then performed (Fig. 1b). Further recording was performed each fourteenth day until the animal was killed.

Histologic technique After killing of the animal fixation of the bone in formaldehyde and decalcification in formic acid the tissue in the chamber space was removed, embedded in paraffin, cut transversally and stained with HTX eosin.

Results and Discussion

The woven bone before irradiation (Fig. 3a) became subsequently lamellated (Fig. 3b). This process involves reorganization of the collagen fibres and the osteocytes of the bone (ALBREKTSSON 1980). In principle, woven bone maturation does not seem to be influenced by the irradiation at least not during the first weeks. At about 4 weeks after the irradiation (Fig. 3c) the mature bone tissue was slightly remodelled as demonstrated by the changed appearance of the islands of connective tissue proper in the bone. This bone remodelling occurred at a similar rate and to the same extent as in normal bone in a series of 70 non irradiated rabbits (ALBREKTSSON 1979). At 6 weeks after injury (Fig. 3d) the vascularity changed and a number of wide venular vessels appeared in the bone tissue. Bone

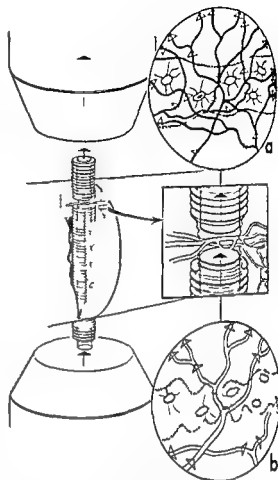


Fig. 1 a) Tissue grown into the chamber space (enlarged view) before irradiation. The rounded drawing is a schematic representation of the chamber microscopic image. b) Osteocyte is thought to represent a nutritive bone unit. c) Intravital recording at 2 weeks after irradiation.

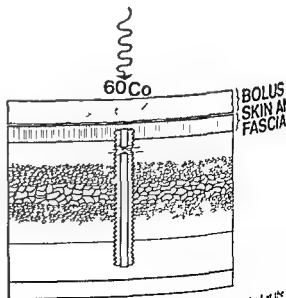


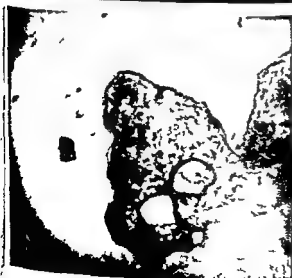
Fig. 2 A 5 mm bolus of silicone rubber was applied at the radiation.



a) Conditions before irradiation: Osteogenesis is indicated by woven bone in some places



b) 2 weeks after irradiation: The former woven bone in the left part of the bone tongue is now mature as indicated by a clear outer demarcation line and normal osteocytes



c) 4 weeks after irradiation all bone in the chamber is considered mature. Up till now the remodeling of the bone is quite normal

Fig. 3 In the explanatory sketch close dots represent mature bone and spaced dots woven or immature bone. Arrows on

vessels indicate direction of blood flow. 15 Gy were given at irradiation



d) 11 week wide venule immature bone appears again and no active bone resorption starts



e) 11 weeks after irradiation bone resorption is widespread and immature bone is further developing



f) 10 weeks after irradiation most of the original bone is resorbed. The chamber image is instead dominated by immature bone with no outer demarcation or a clear representation of lacunae

resorption began. At 8 weeks (Fig. 3 e) resorption of the original bone was more apparent and at 10 weeks most of the original bone was resorbed. The findings of wide venules appearing before beginning of bone resorption should not be considered as implying that the injury to the vessels occurred more rapidly than that to the bone. It has previously been observed (ALBREKTSSON 1980) that bone resorption primarily is associated with wide vessels and slow blood flow. This means that the wider venules observed might just as well reflect a vascular response to abnormal bone as a primary vascular injury due to irradiation. Simultaneously with the onset of bone resorption the development of new bone tissue was obvious (Fig. 3 d right part of the observation field). This new bone tissue rapidly spread over the chamber field during the following weeks (Fig. 4 f). The vital microscopic view of this bone resembled that of woven bone which however normally would become lamellated during a time lapse of 6 weeks. This new bone still lacked definite outer demarcation and clearly established osteocyte lacunae at 10 weeks.

The present experiments have shown that the titanium chamber is a well functioning method for observing and recording bone injury following irradiation. The vital microscopic approach allows a demonstration of the different stages in the development of an irradiation injury. The method has been applied in further experiments on the effects of different types of irradiation injury to bone tissue.

SUMMARY

A vital microscopic method using the titanium chamber for observation of bone irradiation injury *in situ* is described. The subsequent development of bone tissue

resorption and replacement with a pathologic immature bone could be observed and recorded.

REFERENCES

- ALBREKTSSON T. Healing of bone grafts. *In vivo* studies of tissue reactions at autografting of bone in the rabbit tibia. Thesis. University of Gothenburg 1979.
- Repair of bone grafts. A vital microscopic and histologic investigation in the rabbit. *Scand J plast reconstruct Surg* 14 (1980): 1.
- ALGIRE G H. Microscopic studies of the early growth of a transplantable melanoma of the mouse using the transparent chamber technique. *J nat Cancer Inst* 4 (1943): 1.
- CLARK E R and CLARK L L. Microscopic observations on new formation of cartilage and bone in the living mammal. *Amer J Anat* 70 (1942): 167.
- EZRA COHN H E, BULLOUGH P G and TRILETA J. The growth of bone autografts in rabbit ear chambers. *J Bone Jt Surg* 51 B (1969): 372.
- KIEHN C L, CEBUL F, BERG M, GLITENTAG J and GLOVER D M. A study of the vascularization of experimental bone grafts by means of radioactive phosphorus and the transparent chamber. *Ann Surg* 136 (1952): 404.
- KIRBY SMITH II T. Bone growth studies—A miniature bone fracture observed microscopically in a transparent chamber introduced into the rabbit's ear. *Amer J Anat* 53 (1933): 377.
- NATHANSSON A. Bone repair of defects in non irradiated and irradiated mandibles. An experimental study in rabbits. Thesis. University of Stockholm 1977.
- SANDISON J C. A new method for the microscopic study of living growing tissues by the introduction of a transparent chamber in the rabbit's ear. *Anat Rec* 28 (1924): 281.
- A method for the microscopic study of the growth of transplanted bone in the transparent chamber of the rabbit's ear. *Anat Rec* 40 (1928): 41.
- SUDMAN E. Vital microscopy of bone remodelling in rabbit ear chambers. *Acta orthop scand* (1975) Suppl No 160.

THECA-CELL TUMORS

Clinical features and prognosis

ELISABET BJÖRKHOLM and CLAES SILFVERSWÄRD

Theca cell tumors (thecomas) were first described LÖFFLER & PRIESEL (1932). The crude annual incidence among Swedish women in the period 1958-1972 was 0.74 per 100 000 (BJÖRKHOLM & SILFVERSWÄRD 1980). Thecomas belong to the anulus stromal cell tumor group which consists of neoplasms containing granulosa cells, theca cells and stromal cells resembling fibroblasts, singly or in various combinations. Pure theca cell tumors are most always benign (SCULLY 1970, NORRIS & JORLTON 1974), whereas granulosa cell or mixed anulus theca cell tumors may be malignant (NORRIS & SCULLY 1958). Thus it is important to notify patients with pure thecomas who should be treated with surgery alone, not including them in the anulus cell tumor group in which in certain cases radiation may be added. Women with thecomas and granulosa-cell tumors often have endometrial carcinoma (INGRAM & NOVAK 1951, MANSELL & ERTIG 1955). Sixty-two patients with thecoma have been treated at Radiumhemmet; one fifth of them having concomitant endometrial carcinoma. The clinical features and the treatment of these women are now presented. The survival has been determined by comparison with age- and geographically matched control women.

Material and Methods

During the period 1923 to 1972, 313 women with histologically confirmed granulosa stromal cell tumors were treated at this oncologic department.

Material from the ovarian tumor (the original slides or new slides prepared from the original paraffin blocks and stained with haematoxylin-eosin) was still available for 278 cases. A re-evaluation was performed, the clinical data being unknown to the pathologist (C.S.).

The present series ultimately consisted of 62 cases considered to be pure thecomas. Endometrial biopsies dating from time of diagnosis of the ovarian tumor were available for 52 per cent of these cases. The diagnosis thecoma was consistent with the initial diagnosis in 39 cases, 3 cases originally diagnosed as thecoma were now being considered as granulosa cell tumors as they contained epithelial strands of the granulosa cell type, and 17 cases previously diagnosed as granulosa cell tumors were now considered to be thecomas. Six fibromas were at review included in the thecoma group because of typical theca cell proliferation, some of these also with signs of estrogenic function (the patients having glandular cystic hyperplasia of the endometrium). Clinical data were collected from the hospital records. The system of civil registration in Sweden made it possible to obtain age- and geographically matched control women. Limited to the patients residing outside Stockholm City, 40 control women were assigned to 65 per cent (20) of the women with theca cell tumors living outside Stockholm City at

the time of diagnosis. The patient and her 2 controls were of the same age when they entered the series at the time of diagnosis of the patient's ovarian tumor. Information on civil status and childbirth for both groups was obtained. A follow up was performed in 1977 and 1978. A final date for collecting the series was set to 25 November 1977. Causes of death were based on information from death certificates. Survival over time was described with life tables and differences in survival were tested using the log rank test (PETO et coll. 1977).

Results

The mean age at diagnosis was 59.5 years (range 19 to 81 years (Fig. 1) and 84 per cent of the women had passed the menopause. A history of abdominal pain of varying severity was given by one third of the patients. Abnormal uterine bleeding was reported by 6 of 10 premenopausal women and one patient 23 years of age had experienced secondary amenorrhoea during one year before diagnosis. Among postmenopausal women 60 per cent had reported uterine bleeding. All patients belonged to clinical stage I (FIGO). 3 per cent had bilateral tumors and 5 per cent had ascites exceeding 0.5 liter. The mean tumor size was 7 cm (range <1 to 20 cm). Thirteen endometrial carcinomas (21 per cent of the total series) were confirmed at review. 8 were highly differentiated adenomatous carcinoma, 4 were differentiated adenomatous carcinoma with partly solid areas and 1 was a predominantly solid carcinoma (FIGO). The mean age at diagnosis of the women with a concomitant endometrial carcinoma was 69.8 years (range 47 to 71 years). One case of atypical hyperplasia was diagnosed. Glandular cystic hyperplasia was found in 56 per cent of the reviewed endometrial biopsies. The hormonal changes of the endometrium were impossible to observe in one third of the cases as all endometrial tissue was malignantly transformed. The contralateral ovary was available in 12 cases, one third having ovarian stromal hyperplasia. All patients were operated upon (Table). Additional radiation therapy was given to 60 per cent of the patients; of these 16 per cent had a concomitant endometrial carcinoma. At the follow up the mean observation time was 15.5 years (range 1 to 34 years). Thirty-two women had died and no patient was lost to the follow up. No patient with thecoma died from the ovarian neoplasm. Malignant disease caused death in 7 women

PERCENTAGE

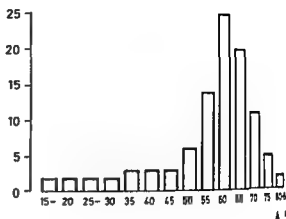


Fig. 1. Distribution of 67 patients with thecoma by age (years).

CRUDE CUMULATIVE SURVIVAL

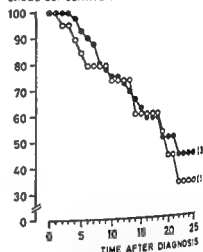


Fig. 2. Crude cumulative survival (per cent) at time after diagnosis (years). The numbers of patients (O) and controls (●) at entry were 20 and 40, respectively. Still living and under observation after 25 years were 1 and 3.

3 endometrial carcinomas, one mammary carcinoma and 3 intestinal tumors.

The crude cumulative percentage of survival (counting deaths from all causes) for the 20 patients with thecoma and their 40 controls is given in Fig. 2. Half of the patients had received radiation therapy, 11 of the 20 patients had a concomitant endometrial carcinoma. No statistically significant difference in survival was found between the patient and the control group. The mortality from cerebrovascular and cardiovascular diseases dominated and was equal for both groups. Endometrial carcinoma caused death in 2 women of the patient group, whereas a control woman died of that cause. Of married women in the patient group 71 per cent had given birth

Table

Sixty-two patients who at review were classified as thecoma by original diagnoses and mode of treatment

Mode of treatment	Original diagnosis			Total No of cases
	Thecoma	Granulosa cell tumor	Fibroma	
Unilat oophorectomy	4	4	0	8
Unilat oophorectomy and radiation therapy	2	1	0	3
Bilat oophorectomy	5	1	3	9
Bilat oophorectomy and radiation therapy	10	6	3	19
Hysterectomy and bilat oophorectomy	5	2	0	7
Hysterectomy bilat oophorectomy and radiation therapy	3	0	0	3
Treatment for concomitant endometrial carcinoma Hysterectomy and bilat oophorectomy with or without radiation therapy	10	3	0	13
Total	39	17	6	62

at least one child at time of diagnosis at that time
corresponding figure for the control group was
per cent

Discussion

A thecoma is generally easy to identify at micro-
scopy. It is composed of pale oval or spindle
shaped cells arranged in irregular interlacing bun-
dles traversed by bands of fibrous tissue with var-
ious degrees of hyalinization. Fat droplets are dem-
onstrable by special stains in and between the
bundles. It may sometimes be difficult to distinguish
thecomas from sarcomatoid granulosa cell tumors.
Granulosa cell areas not clearly identifiable on
hematoxylin-eosin stained slides may be present in
the thecomas (WAXMAN et coll 1979). Silver re-
ticulum stains have been used to try to identify
granulosa and theca cell components. The theca
cells are individually surrounded by this reticulum
whereas granulosa cells are not at all enclosed or as
clumps of cells (TRAUT et coll 1939). The reticulum
on stained slides are sometimes confusing and difficult
to evaluate (KNIGHT 1948) nor do they always re-
veal this network (BIGGART & MACAFEE 1955).
No serious differential diagnostic difficulties were
encountered when reclassifying this series. In one or
two cases a silver reticulum stain might have added
to the diagnostic accuracy but as only the original
slides were accessible and no paraffin blocks a
silver reticulum stain was impossible to perform in
these cases.

It is impossible to draw a sharp demarcation line
between fibromas and thecomas (MORRIS & SCUL-
LY). They may indeed be variants of a single neo-
plasm with a common origin from the ovarian
stroma (AMIN et coll 1971). The thecoma is typi-
cally estrogenic the fibroma is non functioning. In
this series fibromas were defined as small tumors
with markedly few cells of the spindle cell type and
with an abundance of collagen. Fat is usually not
demonstrable in fibromas.

The clinical features of the patients with thecoma
in the present series are similar to those previously
reported (BANNER & DOCKERTY 1945; STERNBERG
& GASKILL 1950). A high proportion of endometrial
carcinomas was found in patients with this tumor.
Feminizing ovarian tumors (granulosa and theca
cell tumors) are often complicated by endometrial
carcinoma. However only 0.4 per cent of the
patients with endometrial carcinoma treated at
Radiumhemmet between 1923 and 1972 had a con-
comitant feminizing tumor. 0.2 per cent were of the
thecoma type (BJORKHOLM unpublished data). Al-
most all thecomas are benign but a few malignant
cases have been reported. A review of the literature
has recently been published by WAXMAN et coll
who proposed that the name malignant thecoma
should not be used. Instead they suggested the name
stromal sarcoma for those very rare malignant
stromal ovarian neoplasms that are composed of
primitive mesenchymal cells, fibroblasts and theca
cells.

No woman in the present series died from the

thecoma. No difference in survival was found between the 20 patients and their 40 controls although more than one third of these patients had a concomitant endometrial carcinoma which in the majority of the cases was highly differentiated. Cerebrovascular and cardiovascular disease was the major cause of death in both patients and controls.

WYNDER *et coll.* (1969) did not find any real differences between patients with ovarian malignancy and controls as regards age at first pregnancy and delivery, last pregnancy and delivery and total number of pregnancies. The number of parous married women was almost the same for the present patients and the controls.

It is noteworthy that half of the patients without endometrial carcinoma received complementary radiation therapy due, no doubt, to a previous uncertainty as to whether these neoplasms were of a malignant nature or not. To day unilateral oophorectomy in premenopausal women with a stage I₁ (FIGO) disease would be suggested. The possibility of a concomitant endometrial carcinoma has to be ruled out especially for postmenopausal women. No complementary irradiation should be given because of the ovarian tumor.

SUMMARY

During the period 1923 to 1972 62 women with theca cell tumors were treated at Radiumhemmet. The mean age at diagnosis was 59.5 years. Concomitant endometrial carcinoma was found in 13 patients. At 1978 after a mean observation time of 15.5 years 30 patients were alive. No patient died from thecoma. Malignant disease caused death in 7 women. No difference in survival was found between 20 patients and 40 controls matched by age and place of residence.

ACKNOWLEDGEMENTS

This investigation was supported by the Swedish Cancer Society and Eugen Froberg's Foundation. The authors would like to thank Mr Bo Nilsson for valuable help with the statistical calculations.

Request for reprints: Dr Elisabet Björkholm, Department of Gynecologic Oncology, Radiumhemmet, S-10401 Stockholm, Sweden.

REFERENCES

- AMIN H K, OKAGAKI T and RICHART R C 1976, A study of fibroma and thecoma of the ovary. An ultrastructural study. *Cancer* 27 (1971) 438.
- BANNER E A and DOCKERTY M B 1964, Theca cell tumors of the ovary. A clinical and pathologic study of thirteen cases (including thirteen new cases) with a review. *Surg Gynec Obstet* 81 (1945) 734.
- BIGGART J H and MACAEEF C H 1965, Tumours of the ovarian mesenchyme. A clinico-pathological survey. *J Obstet Gynaec Brit Emp* 62 (1965) 879.
- BJÖRKHOLM E and SILFVERSWARD C 1980, Granulosa and theca cell tumors. Incidence and occurrence of secondary primary tumors. *Acta radiol Oncology* 19 (1980) 10.
- INGRAM JR J M and NOVAK E 1951, Endometrial carcinoma associated with feminizing ovarian tumors. *Amer J Obstet Gynec* 61 (1951) 774.
- KNIGHT W R 1948, Theca cell tumors of the ovary with a report of fifteen cases and a review of the literature. *Amer J Obstet Gynec* 56 (1948) 311.
- LOFFLER E and PRIESFL A 1932, Bindegewebige Geschwülste des Eierstockes von besonderer Bauart (Fibrom- und thecocelluläre xanthomatöse ovarii). *Beitr Path Anat* 90 (1932) 199.
- MANSELLI H and HERTIG A T 1955, Granulosa theca cell tumors and endometrial carcinoma. A study of their relationship and a survey of 80 cases. *Obstet Gynecol* (1955) 385.
- MORRIS J MCL and SCULLY R E 1969, Endocrine pathology of the ovary. p 65. C V Mosby, St. Louis 1969.
- NORRIS H J and CHORI TON I 1974, Functioning tumors of the ovary. *Clin Obstet Gynec* 17 (1974) 189.
- PETO R, PIKE M C, ARMITAGE P, BRESLOW N E, CHAD D R, HOWARD S V, MANTEL N, MCPHERSON K, PETO J and SMITH P G 1977, Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Brit J Cancer* 35 (1977) 1.
- SCULLY R E 1970, Recent progress in ovarian cancer. *Hum Pathol* 1 (1970) 73.
- STERNBERG W H and GASKILL C J 1970, Theca-cell tumors. With a report of twelve new cases and observations on the possible etiologic role of ovarian stroma hyperplasia. *Amer J Obstet Gynec* 59 (1970) 44.
- TRAUT H F, KUDER A and CADDEE J F 1939, A study of the reticulum and of luteinization in granulosa and theca cell tumors of the ovary. *Amer J Obstet Gynecol* (1939) 798.
- WAXMAN M, VULSTIN J C, URCUYO R and BELL C G 1979, Ovarian low grade stromal sarcoma and thecomatous features. A critical reappraisal of the so-called malignant thecoma. *Cancer* 44 (1979) 224.
- WYNDER E L, DODD H and BARBER H R 1972, Epidemiology of cancer of the ovary. *Cancer* 31 (1972) 352.

SENSITIVITY OF CELLS IN EXPONENTIAL AND STATIONARY GROWTH
PHASE TO COMBINED TREATMENT WITH RADIATION AND QUINACRINE

J. MIDANDER and B. LITTBAND

Quinacrine has been shown to inhibit repair of radiation induced DNA single strand breaks in bacteria and in mammalian cell lines (FUKS & SMITH 1971; MODIG *et coll* 1974; VOICULETZ *et coll* 1974). In the case of bacteria the inhibition of repair could be related to a decreased recovery of the cells from lethal radiation injury but in the case of a mammalian cell line data from survival curves did not indicate such a correlation (VOICULETZ *et coll*). In order to elucidate further the question of a correlation between DNA injury and radiation survival after Quinacrine treatment in addition to investigate the radiation survival of the cells also their recovery during a split dose interval and the survival in a particular low dose range was examined. A quantitative determination of the recovery instead of estimating it indirectly by extrapolation is supposed to give valuable information of the mechanism by which Quinacrine acts as a radiation sensitizing agent. Cells both in exponential and stationary growth phase were used in order to obtain cell material with a varying potential for recovery (JÄRV 1968).

Materials and Methods

A substrain of the Chinese hamster cell line (V79) was used propagated under standard tissue culture conditions. The nutrient medium consisted of Eagle's medium in Earle's saline supplemented with

10 per cent fetal calf serum and antibiotics. For each experiment monodispersed cells derived from 1 day old cultures (cells growing in the exponential phase) or 7 day old cultures (cells growing in the stationary phase) were explanted in known numbers in Anumbra Petri dishes (Ø 5 cm) and incubated in a CO₂ incubator for 3 hours. Quinacrine dihydrochloride (from Sigma Chemical Co.) dissolved in the culture medium was used. In the irradiation experiments treatment with Quinacrine was made as a rule at a concentration of 10 µg/ml for 30 min before and during irradiation. After irradiation the substance was removed by washing the cells twice with trypsin buffer and reincubating them in fresh medium. In split dose experiments Quinacrine treatment was given on both radiation exposures.

The cells were irradiated at room temperature attached to the Petri dishes and with medium removed to such an extent that only a total of one ml was left. The dishes with cover removed were placed in a plastic radiation chamber flushed with pure oxygen supplemented with CO₂ to maintain pH 7.2. After irradiation the dishes were refilled with fresh medium and incubated for 8 days with one medium change on the second day. The clones which developed were fixed and stained *in situ* thereafter the mean number of clones was established by counting three replicate dishes. The frac-

tion of cells which survived irradiation was expressed as the percentage of the non irradiated controls in the same experiment. The plating efficiency was defined as the percentage of non irradiated cells which grow into macroscopic colonies in 8 days.

Radiation was generated with a Siemens Stabilipan Roentgen Unit at 220 kV and 15 mA. The half value layer was 0.3 mm Cu and the dose rate was 3.8 Gy/min at the bottom of the culture dishes at an FD of 40 cm. The dose delivered was measured by a Philips integrating dosimeter.

Results

Toxicity tests In preliminary experiments the cellular toxicity of Quinacrine was analysed with clonogenic survival as end point. One hundred cells from exponentially growing cultures were plated in a series of Petri dishes. After incubation for 3 hours one ml of Quinacrine in different concentrations and dissolved in culture medium was added to the dishes. The cells were incubated with the substance for periods varying between 10 and 120 min, then the cells were washed twice with trypsin buffer and reincubated for 8 days. Fig. 1 indicates that at a concentration of 5 $\mu\text{g/ml}$ Quinacrine had only a slight toxicity up to 60 min, at 10 $\mu\text{g/ml}$ the toxicity was slight up to 30 min, while at higher concentrations (20 and 40 $\mu\text{g/ml}$) severe toxicity was apparent already after 20 min.

Radiation survival curves The effect of Quinacrine (10 $\mu\text{g/ml}$) on the survival of cells in stationary growth phase was analysed in 8 replicate experiments, each paired with Quinacrine untreated controls. Survival determinations were made with 3 different radiation doses which gave surviving fractions below 35 per cent in order to avoid the shoulder region. Quinacrine treatment changed slightly the D_0 but no change occurred in the extrapolation number (n) of the survival curves (Fig. 2). In 6 similar replicate experiments with cells in the exponential growth phase and irradiated with 4 different doses, a slight change in the D_0 was noted but a considerable decrease of n (Fig. 3).

The data presented in Figs 2 and 3 suggest that Quinacrine in the concentration used inhibits the recovery from sublethal radiation injury in cells in their exponential growth phase. Cells in their stationary growth phase known to have a reduced capacity

SURVIVING FRACTION

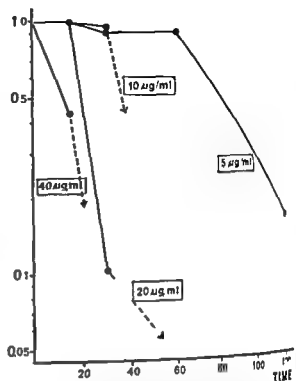


Fig. 1 Survival of Chinese hamster cells after exposure to Quinacrine in different concentrations for varying times. Means are illustrated from triplicate determinations.

to recover (HANSEN 1968) are not detectably inhibited by Quinacrine.

Low dose experiments The conclusion drawn was confirmed in 2 types of complementary experiments. In one of them cells were irradiated with relatively low doses to obtain survival in the shoulder region of the survival curves. In 9 paired experiments performed with cells in the exponential growth phase and in 7 paired experiments with cells in the stationary phase the survival of Quinacrine-treated and Quinacrine untreated cells was determined and the survival ratio calculated. The survival ratio for the cells in the exponential phase increased from a value close to unity up to a value near 2 with doses from 0.65 to 2.60 Gy (Fig. 3). For cells in the stationary phase only a slight increase was noted from unity to about 1.2.

Split dose experiments In another series of replicate experiments the split dose ratio of Quinacrine treated and Quinacrine untreated cells was calculated. The cells were irradiated either with a single dose of 7.80 Gy or with 3.90 Gy on 2 occasions separated by an interval of 18 hours. Previously this time was

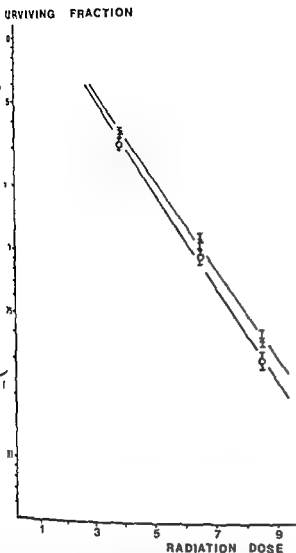


Fig. 2 Survival of cells irradiated (Gy) in the stationary growth phase after treatment with 10 μ g/ml Quinacrine. Means \pm SE are plotted from 8 replicate Quinacrine treated experiments (O) and 8 replicate Quinacrine untreated controls (X). Regression lines were calculated by least square analysis of the pooled data. D_0 was 1.94 and 2.05 Gy and n was 2.47 and 2.47 for the Quinacrine treated and Quinacrine untreated cells respectively.

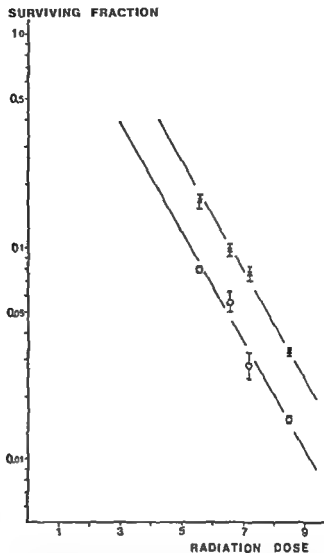


Fig. 3 Same as Fig. 2 but the cells were irradiated in the exponential growth phase. D_0 was 1.69 and 1.79 Gy and n was 2.3 and 3.8 for the Quinacrine treated (O) and Quinacrine untreated (X) cells respectively.

found to be less than the time necessary to complete cell division under the conditions used (LITTBAND 1970). Only a slight decrease in the yield of cells in the stationary phase occurred during this time interval (Fig. 5). In contrast Quinacrine inhibited considerably the recovery in the exponential phase cells.

Discussion

The 3 types of irradiation experiments are consistent in indicating that recovery of the cells from lethal radiation injury is inhibited by Quinacrine

when the cells were treated in their exponential growth phase exhibiting a large recovery potential. When the cells had been treated in their stationary phase of growth having a reduced recovery potential no or only a slight statistically not significant inhibition of recovery by Quinacrine occurred. A difference between the recovery processes in exponentially growing and stationary phase cells, one process being more affected by Quinacrine than the other, can be one explanation for the observation. This explanation is supported by calculations which indicated that the extrapolation number (n) of the survival curves which can be regarded as an ex-

SURVIVAL RATIO

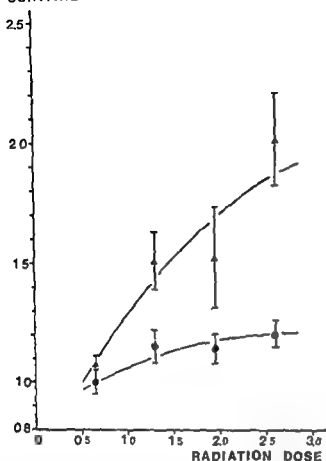


Fig. 4 Survival ratio of Quinacrine untreated to Quinacrine treated cells after irradiation (Gy) within a low dose region. Means \pm SE are illustrated from replicate experiments with cells in their exponential (Δ 9 paired experiments) or stationary (\bullet 7 paired experiments) growth phase.

SPLIT DOSE RATIO

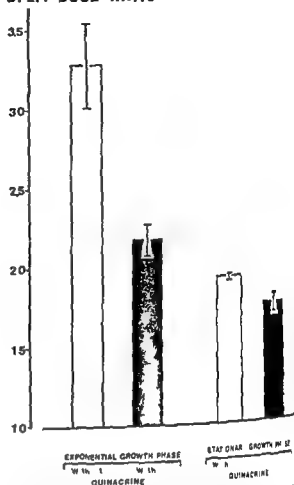


Fig. 5 Split dose ratio of Quinacrine treated and Quinacrine untreated cells after irradiation with 2×3.9 and 1×7.8 Gy. Columns illustrate Means \pm SE from replicate experiments with cells in their stationary (8 paired experiments) or exponential (4 paired experiments) growth phase.

pression for the recovery potential of the cells in the case of exponentially growing cells (about 3.8) was reduced to almost the same value (about 2.3) as in the case of stationary phase cells (about 2.5). In agreement with this finding the split dose ratio for the exponentially growing cells is also reduced to about the same value as the ratio for the stationary phase cells (around 2). Alternatively the difference between the effect of Quinacrine on the cells in the 2 phases of growth can be explained also by the standard deviation of the differences in the 2 cases: the significance of small differences for stationary phase cells being more difficult to evaluate statistically.

Working with two strains of *E. coli* one possesses an efficient repair system for radiation induced DNA single strand breaks and the other deficient of

Thus their results suggest a relationship between DNA injury and survival. Such a relationship is suggested also by the present results in consideration of previous observations (MODIG *et al.*) made with the present Chinese hamster cell line and which indicated an inhibition of the repair of DNA single strand breaks by Quinacrine. On the other hand VOICULETZ *et al.* argue that no relationship exists since they did not find any difference in the extrapolation number of the survival curves of Quinacrine treated and Quinacrine untreated Chinese hamster ovary cells. This argument based upon negative observations needs further experimental support from more direct tests e.g. split dose experiments or experiments in which

ent of malaria and other infectious diseases. This experience could be of a great value if Quinacrine is of any advantage in radiation therapy. At present only few reports on the sensitizing effect of Quinacrine on experimental tumors *in vivo* have appeared (ACKERMAN *et coll.* 1965, ALGHILATI 1966). It is conceivable that Quinacrine is useful in the treatment of tumors which have a higher potential to recover from radiation injury than normal tissues, e.g. in malignant melanoma (DEWEY 1971). The present data suggest further investigations in this respect.

SUMMARY

The effect of Quinacrine on the recovery of cells from sublethal radiation injury was analysed by testing cellular survival after exposure to low and high radiation doses and after irradiation with split doses. Quinacrine was found to inhibit recovery in cell cultures in exponential growth phase but not or only slightly in cells in stationary growth. This finding is related to the inhibitory effect of Quinacrine on the repair of radiation induced DNA strand breaks.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the valuable discussions with Prof. L. Revesz. Skilful technical assistance was provided by Mrs Ingegerd Hedlöf and Mrs Rut

Jonsson. The investigation was supported by grants from the Swedish Cancer Society.

Request for reprints: Dr J. Midander, Department of Tumor Biology II, Karolinska Institutet, S-104 01 Stockholm, Sweden.

REFERENCES

- ACKERMAN N. H., HALDORSEN D. & WALLACE D. L., MADSEN A. J. and MCFEE A. S. Aminoacridine uptake by experimental tumors. *J. Amer. med. Ass.* 191 (1966) 115.
- ALGHILATI L. J. Uptake of iodine 131 labeled Atabrine by Ehrlich ascites tumor and by sarcoma S 180 BALB. *Nature (London)* 211 (1966) 878.
- DEWEY D. L. The radiosensitivity of melanoma cells in culture. *Brit. J. Radiol.* 44 (1971) 816.
- FLASZ Z. and SMITH K. C. Effect of Quinacrine on X-ray sensitivity and the repair of damaged DNA in *Escherichia coli* K 12. *Radiat. Res.* 49 (1971) 63.
- HAN G. M. Failure of Chinese hamster cells to repair sublethal damage when X irradiated in the plateau phase of growth. *Nature (London)* 217 (1968) 741.
- LITTELAND B. Survival characteristics of mammalian cell lines after single or multiple exposures to roentgen radiation under oxic or anoxic conditions. *Acta radiol. Ther. Phys. Biol.* 9 (1970) 257.
- MODIGH G., EDGREN M. and REVESZ L. Dual effect of oxygen on the induction and repair of single strand breaks in the DNA of X irradiated mammalian cells. *Int. J. Radiat. Biol.* 6 (1974) 341.
- VOHLLETZ N., SMITH K. C. and KAPLAN H. S. Effect of Quinacrine on survival and DNA repair in X irradiated Chinese hamster cells. *Cancer Res.* 34 (1974) 1039.

CALCITONIN AND MAMMARY CARCINOMA

LENNART WAHLBY and GUNNAR WESTMAN

Ectopic production of hormones by neoplasms is a relatively unknown subject that may offer diagnostic possibilities. The calcitropic hormone calcitonin is known to be produced by the C cells of the thyroid gland. The plasma calcitonin is usually increased in patients with medullary thyroid carcinoma (DEFTOS 1978). Increases in plasma calcitonin have also been found in patients with other endocrine malignancies, e.g. oat cell carcinoma of the lung and breast carcinoma (SILVIA et coll 1974, COOMBES et coll 1976). Especially when dealing with metastatic breast carcinoma, the plasma calcitonin level is reported to be increased in a majority of patients (COOMBES et coll 1976, OLSEN et coll 1978). An evaluation has been performed to elucidate whether determination of immunoreactive plasma calcitonin could be of any value in the preoperative examination of patients with breast carcinoma and whether calcitonin thus could be named a tumor marker. The results are now reported.

Material and Methods

The material consisted of 33 women, mean age 56.8 years (range 36–88). Clinical examination, scintigraphy of the liver and skeleton and microscopic examination of the breast specimen revealed no metastases in 13 patients.

Ten women had regional lymph node metastases and another 10 had known bone or other metastases. Blood samples were obtained for determination of calcium, phosphorus, creatinine and calcitonin. Calcitonin was determined in serum by the radioimmunoassay method described by ALMQVIST et coll

(1974). All determinations were made twice. The coefficients of variation were 18 per cent. The calcitonin levels were classified as normal or elevated.

Results

All patients except 3 had normal plasma calcitonin levels (Table 1). Two of the 3 patients with elevated calcitonin had axillary lymph node metastases and bone metastases. Furthermore, one of these two women had large metastases in the liver and in both ovaries. Eight of the patients (24 per cent) had bone metastases but only 2 of these had elevated plasma calcitonin. Osteolytic bone metastases were found in 5 of these 8 women but only 2 of them had elevated plasma calcitonin. All 3 patients with elevated plasma calcitonin had normal serum calcium and phosphorus as well as creatinine.

Discussion

Several authors (COOMBES et coll 1975, OLSEN et coll 1978, RASMUSSEN et coll 1978) have found elevated plasma calcitonin in patients with breast carcinoma and particularly when distant metastases were present (Table 2). However, in the present series no correlation between plasma calcitonin level and women with breast carcinoma existed even in the presence of bone metastases.

The absence of correlation in the present series might be due to the fact that in the preparation of antibodies to human calcitonin, an extract from medullary carcinoma of the thyroid was used.

(ALMQVIST *et coll*.) This may produce antibodies of another aminoacid composition than the corresponding calcitonin antibodies produced by breast carcinoma tissue. This suggestion is made as calcitonin from different species can differ in the aminoacid structure (DEFTOS). Such immunoheterogeneity has been further proved by ROOF *et coll* (1979) in a series of patients with different types of malignant disease. However, high molecular weight immunoreactive calcitonin has been found in the plasma of patients with medullary carcinoma of the thyroid as well as in the medias in which breast carcinoma cells or cells from medullary carcinoma of the thyroid have been cultured (COOMBES *et coll* 1976, DEFTOS, GOLTZMAN & TISCHLER 1978). This may indicate that the calcitonin antibodies should react towards calcitonin of both types of tissues.

The method used differs in one point from the method used by OLSEN *et coll* who found a high correlation between calcitonin and breast carcinoma. They used synthetic calcitonin instead (DIETRICH *et coll* 1975). However, this should not be responsible for the difference as mentioned.

Another reason for the poor correlation may be that calcitonin is not produced in the patients until bone metastases, and mainly osteolytic metastases, have appeared. OLSEN *et coll* found that plasma calcitonin was frequently elevated in patients with osteolytic metastases. However, in the present material 5 of 8 patients had metastases of this type but only 2 of them had elevated plasma calcitonin. The elevation of calcitonin in these patients could be a secondary effect and produced by the C cells of the thyroid gland as a result of hypercalcemia due to osteolytic metastases. Recently JORGENSEN *et coll* (1979) reported on 137 patients with breast tumours. They could not prove any increase in immunoreactive calcitonin in either benign or malignant tumors or the adjacent tissue. This is further emphasized in the present series, and a similar conclusion can be drawn from the results of RASMUSSEN *et coll*.

It may thus be concluded that the determination of plasma calcitonin is of no value in the evaluation of patients with breast carcinoma and it should not be referred to as a tumor marker of breast carcinoma.

SUMMARY

Plasma calcitonin was determined in 33 patients with breast carcinoma. Eight patients had bone metastases as revealed at scintigraphy and radiography. Only 3 of the 33

Table 1

Calcitonin in patients with breast carcinoma with or without visible metastases

	Normal	Raised
No metastases	1*	1
Regional lymph node metastases	10	0
Bone metastases (osteolytic bone metastases)	8 (5)	7 (1)
Total	30	3

Table 2

Different series of plasma calcitonin in breast carcinoma

	Number of patients with elevated plasma calcitonin	
	With or without regional metastases	With bone metastases
COOMBES <i>et coll</i> (1975)	4/30	2/9
OLSEN <i>et coll</i> (1978)	6/71	10/13
RASMUSSEN <i>et coll</i> (1978)	0/8	1/6
Present series	1/73	7/10

patients had elevated plasma calcitonin. Two of these had osteolytic bone metastases. It is concluded that the determination of plasma calcitonin is of no value in the evaluation of patients with breast malignancy and it should not be referred to as a tumor marker.

REFERENCES

- ALMQVIST S, TELNIUS BERGM and WASTHED B. Serum calcitonin in medullary thyroid carcinoma. *Acta endocrinol* 196 (1974) 1977.
- COOMBES R C, EASTY G C, DILLER S I, HILLIARD J, SYLVANS U, GIRCIS S I, GALANTE L S, HAYWOOD L, MACINTYRE I and NEVILLE A M. Secretion of immunoreactive calcitonin by human breast carcinomas. *Brit med J* 4 (1975) 197.
- ELLISON M L, EASTY G C, HILLIARD C J, JONES H, GALANTE L, GIRCIS S, HAYWOOD L, MACINTYRE I and NEVILLE A M. The ectopic secretion of calcitonin by lung and breast carcinomas. *Clin Endocrinol* 5 (1976) 387 S.
- DEFTOS L J. Calcitonin in clinical medicine. *N Engl J intern Med* 23 (1978) 159.
- DIETRICH F M, HUNZIKER W H and FISCHER J A. Synthetic human calcitonin. *Acta endocrinol* 80 (1976) 465.
- GOLTZMAN D and TISCHLER A S. Characterization of

- the immunochemical forms of calcitonin released by a medullary thyroid carcinoma tissue culture *Clin Invest* 61 (1978) 449
- ØRGENSEN O G, EKELAND A and GAUTVIK K M. Serum and tissue concentrations of immunoreactive calcitonin in patients with breast tumours *Acta endocr* 92 (1979) 522
- ØLSEN K J, GADEBERG C, NIELSEN H E and JOHANSEN A. Increased serum calcitonin in patients with mammary carcinoma *Acta radiol Ther Phys Biol* 17 (1978) 263
- RASMUSSEN B, ROESDAHL K and LINDGREEN P. Parathyroid hormone and calcitonin in serum of patients with mammary carcinoma *Acta radiol Ther Phys Biol* 17 (1978) 269
- ROOF B S, WEINSTEIN R, VUJIC I and BERDASH N M. Immuno-heterogeneity of the calcitonins of hypercalcemia: breast and lung cancers and medullary carcinoma of thyroid *Biomedicine* 30 (1979) 82
- SILVER O L, BECKER K L, PRIMACK A, DOPPMAN J and SNIDER R H. Ectopic secretion of calcitonin by oatcell carcinoma *New Engl J Med* 290 (1974) 1122

EFFECT OF A SCREENING PROGRAMME ON BREAST CARCINOMA
INCIDENCE MORTALITY AND SURVIVAL

IRMA SOINI and MATTI HAKAMA

The malignant cases detected in screening are an efficient basis for an evaluation of the program.

The incidence and mortality of the screened population compared with the non screened population provide a better indication of the efficacy of the programme. Primary results of screening for breast carcinoma i.e. the numbers of malignant cases detected have been amply reported but the follow up of the women screened has often been incomplete and of short duration 10 years at the longest (MILNER 1978). In some programmes health education on self-examination of the breast has also been given (ANGELAND 1970 DAVEY et coll 1974) but no results on its effect on the subsequent risk of breast carcinoma have been reported.

The local Cancer Society in one Finnish city organized a breast carcinoma screening programme in 1974 to 1975 with special attention paid to health education (SOINI & LAUSLAHTI 1976). The effect of the programme on incidence mortality and survival will be given in the present report.

Material and Methods

All women aged 41 to 60 years (i.e. all women born in 1914-1933) in Tampere an industrial city in central Finland were invited to attend the screening programme during the period April 1974 to March 1975. In 1974 Tampere had a population of 167 750 including 20 644 females in the age group invited. In the screening the breasts and the axillary lymph nodes were examined and palpated by specially trained nurses. Women with abnormal findings were referred to a physician for further examination. The

indications for this further examination were a lump retracted nipple secretion change in the skin of the breast and the nipple big breasts difficult to palpate pain in the breasts and enlarged axillary lymph nodes. The women were also instructed in monthly self-examination and given an illustrated leaflet published by the Cancer Society of Finland with further instructions.

Further examinations by a physician included palpation and if indicated fine needle biopsy or secretion specimen mammary radiography and thermography. A woman with abnormal findings requiring no surgery was asked to undergo a new examination at a later date. Patients who were referred for surgery as a result of the palpation radiography and thermography or cytologic examination were operated upon at the local City Hospital.

The follow up of the screened population (ad 30.6.1978) was based on the figures of the local histologic laboratories (incidence and stage of carcinoma) on the death certificate reports of the Office of the City Physician (mortality) and on the records on individuals of the group in the National Population Registry (emigration). Only women who did not move from the area were followed in 1974 to 1978 because the follow up of the total group would have caused a considerable delay in the evaluation of the results.

New cases of and deaths from breast carcinoma among females aged 41 to 60 in Tampere in 1960 to 1973 were found from the original records of the Finnish Cancer Registry. The Finnish Cancer Regis-

try is a population based registry which covers the entire country. All hospitals, pathologic and cytologic laboratories and practitioners have been requested to make reports to the Finnish Cancer Registry of all new cases of malignant tumours since 1953. The registry receives a copy of every death certificate in which mention is made of malignant disease. The files of the Finnish Cancer Registry are cross checked annually against the lists of all deaths occurring in Finland.

The incidence and mortality figures in this report for breast carcinoma for 1960 through 1973 were cross sectional rates by calendar period for women in Tampere aged 41 to 60, whereas the estimates of incidence and mortality for the screened population in 1974 to 1978 were rates for women born 1914 to 1933. The observed rates in 1974 to 1978 were compared with those expected from extrapolation of the linear trend in 1960 to 1973.

Survival rates were calculated by the actuarial method (BERKSON & GAGE 1950). The malignant cases were divided into three groups: those diagnosed in 1960 to 1963, 1970 to 1973 and 1974 to 1977, according to whether they were diagnosed before, during or after the screening programme. The patients diagnosed in 1960 to 1963 and 1970 to 1973 were followed up to the joint closing date of 31.12.1974 through the Finnish Cancer Registry, whereas the most recent group was followed up to 31.12.1977 by means of data from the Office of the City Physician. Consequently the follow up periods were 12 to 15, 2 to 5 and 0 to 4 years, respectively. Deaths from causes other than breast carcinoma were found only in the group diagnosed in 1960 to 1963 (9 cases). The deaths were treated as withdrawals alive when the survival rates were estimated. In this case the method gave the same result as that based on competing risks of death (CHIANG 1968).

Results

Of the 20644 women invited to attend the screening programme, 17261 (84%) actually did so. The examining nurses referred 627 (3.6%) women for further examination, which was performed in 615. Of these 615, 117 (19.7%) per cent of the entire series were referred to the hospital for surgery. Twenty seven had malignant tumours and 90 benign lesions. Four of the malignant cases were non-infiltrative intraductal carcinomas and seven were preclinical

Table 1

The stage of new cases of breast carcinoma in Tampere in 1978. Women aged 41 to 60 years in 1960 to 1973, women born 1914 to 1933 in the screened population in 1974 to 1978

Year	Total No of cases	Stage unknown		Stage known	
		Number	Per cent of all cases	Number	Per cent of all cases
1960	20	0	0	20	100
1961	24	1	4.2	23	95.8
1962	18	5	27.8	13	69.4
1963	17	5	29.4	12	70.6
1964	11	2	18.2	9	81.8
1965	14	2	14.3	12	85.7
1966	25	4	16.0	21	84.0
1967	15	6	40.0	9	60.0
1968	12	2	16.7	10	83.3
1969	24	2	8.3	22	91.7
1970	18	1	5.6	17	94.4
1971	28	0	0	28	100
1972	25	1	4.0	24	96.0
1973	27	1	3.7	26	96.3
1974	29	0	0	29	100
1975	28	0	0	28	100
1976	19	0	0	19	100
1977	23	0	0	23	100
1978	10	0	0	10	100

Table 2

New cases of breast carcinoma and deaths in 1960 to 1978, women aged 41 to 60 years in Tampere

Year	Person years at risk	New cases		Deaths	
		Number	per 10 ⁵	Number	per 10 ⁵
1960	19 200	0	1.04	11	0.68
1961	19 330	24	1.24	14	0.72
1962	19 460	18	0.92	9	0.46
1963	19 590	17	0.87	8	0.41
1964	19 720	11	0.56	10	0.51
1965	19 850	14	0.71	5	0.25
1966	19 980	25	1.25	4	0.20
1967	20 110	15	0.75	7	0.35
1968	20 240	17	0.84	9	0.44
1969	20 370	24	1.19	10	0.49
1970	20 507	18	0.88	8	0.39
1971	20 531	28	1.36	10	0.49
1972	20 578	25	1.22	9	0.44
1973	20 597	27	1.31	9	0.44

Table 3

breast carcinoma and deaths in the screened population in 1974 to 1978 for women in Tampere born in 1914 to 1933

Year	Person years at risk	New cases		Deaths	
		Number	per 10 ³	Number	per 10 ³
1974	70 644	29	1.40	9	0.44
		13			
		16			
1975	70 077	28	1.39	9	0.45
		14			
		14			
1976	19 593	19	0.97	8	0.41
1977	19 305	23	1.19	5	0.26
1978	9 537	10	1.05	2	0.21

In the screening
Outside the screening

on palpable carcinomas. Two cases were diagnosed among the 615 women who attended the further examinations during the follow up period 1975 to 1978 and one of them was found in the group (36 cases) referred for closer surveillance by the physician. Localized carcinomas detected in the screening accounted for 55.6 per cent of the total. The figure was 41.4 per cent of all diagnosed malignant tumours in 1974 and 60.7 per cent of those in 1975. The percentages of localized carcinomas year by year from 1960 to 1978 are presented in Table 1.

During the period 1960 to 1973, 11 to 28 new breast carcinoma cases and 4 to 14 deaths from breast carcinoma were registered corresponding to an incidence of 0.6 to 1.4 per 10³ and mortality 0.2 to 0.7 per 10³ annually (Table 2).

In 1974, 16 and in 1975, 14 further malignant cases were detected outside of the screening programme in the female population invited to participate in the programme in addition to the 27 new breast carcinoma patients diagnosed on the basis of screening. Consequently, the total incidence in 1974 was 1.40 per 10³ and in 1975, 1.39 per 10³. The trends of incidence and mortality during and after screening are presented in Table 3. The screening was associated with a slight increase in incidence in 1974 to 1975 and a slight decrease during follow up period. The mortality figures decreased during the total period after screening.

The expected incidence and mortality figures in 1974 to 1978 were calculated from the linear trend in

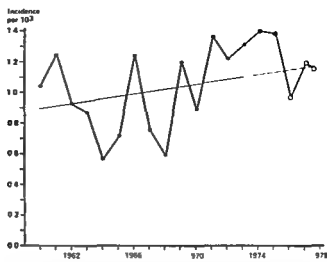


Fig. 1. Observed and expected breast carcinoma incidence rates in 1960 to 1978 in Tampere. Cross sectional rates in 1960 to 1973 for women aged 41 to 60 years; group rates in 1974 to 1978 for women born in 1914 to 1933. During screening (●). After screening (○).

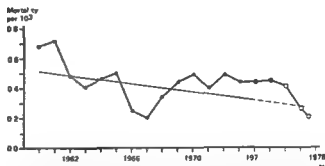


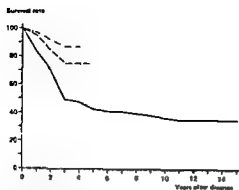
Fig. 2. Observed and expected breast carcinoma mortality rates in 1960 to 1978. Cross sectional rates in 1960 to 1973 for women aged 41 to 60 years; group rates in 1974 to 1978 for women born in 1914 to 1933. Residents of Tampere at the time of diagnosis. During screening (●). After screening (○).

1960 to 1973. A comparison of the observed figures with those expected reveals no substantial change during and after the screening programme (Figs 1 and 2).

The survival curves of the patients with carcinoma diagnosed in 1960 to 1963, 1970 to 1973 and 1974 to 1977 are presented in Fig. 3. The rates increased by the year of diagnosis and the difference in 4 year rates between patients diagnosed in 1960 to 1963 and 1974 to 1977 was 40 per cent.

Discussion

After effective screening the incidence of the disease should be lower during the next few years than during the actual screening period. The successful



Observed survival curves (in per cent) of breast carcinoma for the periods 1960 to 1963 (—) 1970 to 1973 (---) and 1974 to 1977 (— · —) (all women born in 1914 to 1933) Residents of Tampere at the time of diagnosis

therapy of early malignant cases detected in the screening should cause a decrease in mortality and an increase in survival. An early diagnosis should also result in an increased proportion of localized cases. The incidence of and mortality from breast carcinoma are the most reliable indices for evaluating the efficacy of a screening programme. There is evidence that screening for breast cancer decreases breast cancer mortality (MILLER 1978).

Because the screening programme should detect preclinical early cases, the incidence of new cases in the screening consists of normal incidence during the screening period and of the prevalence of preclinical malignant cases in the screened population. The number of preclinical cases (7/27) in the present screening programme was small. This result is likely to be due to the primary screening method which was only palpation. A preclinical tumour is non palpable by definition. Consequently only some of the preclinical cases were likely to be detected in the screening.

A high risk group of 627 females from the screened population of 17261 women was identified in the clinical examination. Besides tumours confirmed in the screening, only 2 malignant cases were detected during the follow up period in this group. Accordingly the screening was also an inadequate method of defining a high risk group. This is in agreement with the result that benign lesions are independent of the risk of breast malignancy (SOINI & HAKAMA 1979).

The increase in the incidence during screening as well as the decrease after screening were almost non-existent (Tables 2-3). The deviations of incidence and mortality from the expected figures estimated on the basis of the trend in previous years

(1960 to 1973) were also insignificant (Figs 1-2). The improvement in survival rates by calendar period has continued and there was no evidence that the survival rates of the patients diagnosed during screening were better than expected on the basis of long term trends (Fig. 3). Further thanks to screening early detection apparently lengthens the survival time through the length bias (ELEN 1976) and survival rates do not provide a reliable basis for the evaluation of the screening.

The proportion of localized carcinomas in the screened population should be higher than in the non screened one. In this screening the proportion of localized carcinomas was higher than in the previous years but did not deviate from the general trend. The fact that the proportion of localized tumours had generally increased can be accounted for by change in the biologic type of the disease or a change in diagnostic criteria, health education or the combined effect of all these factors.

All immediate indices of the efficacy of the screening, higher frequencies of localized cases, improved survival, decreases in incidence and mortality within the screening period (1974 to 1975) was compared with the subsequent years (1976 to 1978) would indicate that screening was beneficial. However, consideration of longer trends raised serious doubts as to any major effect the screening programme might have on the risk of malignant tumours of the breast. The results can be accounted for by factors other than the screening. Changes in incidence, mortality, survival and the proportion of localized cases essentially followed the expected long term trend. Furthermore the improved survival rates were affected by the length bias and did not give any reliable basis for an evaluation of the screening. The present results emphasize the need for a properly defined control group. Proper controls are found if the screening programmes follow an experimental design when the effects of screening on the risk of disease are evaluated.

SUMMARY

Screening for breast carcinoma revealed 77 cases of malignancy, 7 of them preclinical. The method consisted of palpation and health education. Mammography and fine needle biopsy were used in uncertain cases. Shortly after the screening the incidence and mortality were lower (1.07 and 0.29 per 10⁵ respectively) than during the screening (1.40 and 0.45 per 10⁵). Survival after the screening was higher (1.40 and 0.45 per 10⁵) than during the screening (1.40 and 0.45 per 10⁵). Survival after the screening was higher (1.40 and 0.45 per 10⁵) than during the screening (1.40 and 0.45 per 10⁵).

patients diagnosed during or after screening than for patients diagnosed before. However, the changes did not show any substantial differences from the long term trends. It was concluded that the screening programme had no real effect on the malignancy risk. The absence of effect was accounted for by the relatively inefficient screening method.

Request for reprints: Dr Irma Soini, Department of Radiology, Turku University Central Hospital, SF-20520 Turku 52, Finland.

REFERENCES

- ERKSON J and GAGE III R. Calculation of survival rates for cancer. *Mayo Clin Proc* 25 (1950) 270.
- TEANG C L. Introduction for stochastic processes in biostatistics. John Wiley and Sons, New York, 1968.
- DAVEY J, PENTNEY B H and RICHTER A M. The early diagnosis of breast cancer. *Practitioner* 213 (1974) 365.
- LANGELAND P. Population screening for female breast tumours. A clinical investigation. *Acta radiol* (1970) Suppl. No. 297.
- MILLER A B (editor). Screening in cancer. UICC Technical Series Vol. 40. UICC, Geneva, 1978.
- SOINI I and HAKAMA M. Inverse association between risk factors for benign and malignant breast lesions. *Scand J soc Med* 7 (1979) 79.
- and LAUSLAHTI K. Screening for breast cancer in women aged 41–60. *Ann clin Res* 8 (1976) 403.
- ZELEN M. Theory of early detection of breast cancer in the general population. In: *Breast cancer: Trends in research and treatment*, p. 287. Edited by J C Henson, W H Matthei and M Rojensweig. Raven Press, New York, 1976.

EFFECT OF SINGLE DOSE IRRADIATION ON THE PROLIFERATION KINETICS IN A HUMAN MALIGNANT MELANOMA IN ATHYMIC NUDE MICE

E. K. RØFSTAD, T. LINDMO and T. BRUSTAD

Design of optimum fractionation regimes in radiation therapy requires among other factors detailed information on the kinetics of cell death and cell proliferation after each fraction. The proliferation kinetics in a considerable number of non irradiated animal and human tumours and normal tissues have been reported (for review cf. STEEL 1977) but the effect of irradiation on the proliferation kinetics has been analysed only for a few types of tissue. However partial cell synchrony shortly after irradiation due to mitotic delay or selective inactivation of cells in the most sensitive phases of the cell cycle has been demonstrated for both tumours and normal tissues (HALLMAN et coll 1966, WITHERS & ELND 1969, DENEKAMP et coll 1969, ROCKWELL & CALLMAN 1974). The cell cycle time of cells of normal tissues has been shown to be shortened during regrowth (CAIRNIE 1967, LESHER 1967, BROWN & BERRY 1969, BROWN 1970) but results from tumours during regrowth are conflicting. Both shortened, prolonged and unchanged cell cycle times as well as increased and reduced growth fractions have been reported (TUBIANA et coll 1968, TUBIANA 1971, BROWN & BERRY, BROWN, HERMENS & BARFØDSEN 1969, 1970, 1975, FRINDEL et coll 1970, DENEKAMP & THOMLINSON 1971, VAN PEPERZEEL 1972, SZCZEPANSKI & TROTT 1975, KOVACS et coll 1976, NELSON et coll 1976, BRAUNSCHWIEGER et coll 1979).

Further investigations of these problems seem to

be needed particularly of experimental tumours which may be representative models for human tumours. Thus the cells of human tumours grown in immune deficient animals have been shown to have phase durations in the range of those found for cells of human tumours in man (PICKARD et coll 1975, RØFSTAD et coll 1977a). Therefore the effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma grown in the athymic mutant nude mouse was analysed. The results are discussed in relation to the radiation response of the tumour as determined from measurements of single cell survival and tumour size.

Materials and Methods

Young male nu/nu/BALB/c/BOM mice were used. The animals were kept under conventional conditions in a special room at 27°C with automatically regulated 12 hour periods of light and darkness.

The malignant melanoma (E.E. malignant melanoma) was taken from a lymph node metastasis in the left axilla of a 62 year-old man. The tumour tissue was composed of melanin poor atypical nevus cells growing in large balls. Cells and nuclei varied greatly in size and shape. Innumerable mitoses were seen.

Passages 16-22 were used. The histologic appearance of the tumour had not changed after 22 passages in nude mice. Calipers were used to measure the length of two perpendicular axes of the tumours and the cross sectional area of the tumours calculated as being elliptical was used as a parameter for tumour size. The cross sectional area of the tumours ranged from 50 to 100 mm² on the day the experiments were initiated.

Irradiation procedure. The mice were irradiated locally without anaesthesia at a focus skin distance of 80 cm by applying one tangential radiation field from a 5000 Ci (1.85×10^{14} Bq) ⁶⁰Co therapy unit (F.M. Mobaltron 80). Acceptable dosimetric conditions were obtained by immersing the mice partly in a water phantom whereby the tumours were positioned under water and at a distance from the outer surface of the phantom wall greater than that corresponding to the ionization maximum of the radiation used. Perspex tubes served as mouse holders. The water temperature was kept at 30°C. Measurements made with thermoluminescent discs (TLD 100 LiF Ribbon Harshaw) placed at the ionization maximum varied within a few per cent of the corresponding ionization chamber readings of 1.4 Gy/min. Further details of the irradiation procedure are described elsewhere (ROFSTAD et al. 1977b).

Flow cytometry. The tumours were finely minced in phosphate buffered saline by means of a scalpel and a pair of tweezers and the tissue suspensions were filtered through 100 µm filters (Nytal Schweizersche Seidengazefabrik AG). Cell debris and injured cells were separated from the intact cells in a filtered cell suspension by a centrifugation technique based on the same principles as those described by SEGLEN (1976) for rat liver cells. The cell suspension was layered over a 15 per cent metrizamide cushion and after centrifugation at 1000 rpm for 5 min most of the injured cells were sedimented to the bottom of the tube while the intact cells were layered at the interface. The supernatant containing cell debris was removed and after a second centrifugation at 2000 rpm for 5 min a clean cell suspension was obtained. The cells were stained with mithramycin without previous fixation (CRISSMAN & TOBEY 1974) and analysed by flow cytometry. The 457 nm line of a 4 W argon laser was used for excitation of the mithramycin fluorescence which was detected at wavelengths longer than 476 nm. DNA histograms were regis-

tered in 128 channels of the multichannel analyzer of the flow cytometer (LINDMO & STEEN 1979; LINDMO & PETTERSEN 1979).

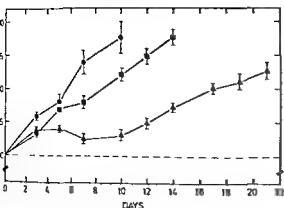
In vitro colony assay. A soft agar colony assay based on the technique described by COURTENAY (1976) and COURTENAY & MILLS (1978) was adapted for E.E. malignant melanoma. The culture medium used was Ham's F12 medium with 10% foetal calf serum, 250 mg/l penicillin and 50 mg streptomycin (Gibco Biocult).

Single cell suspensions were prepared from the tumours without the use of enzymes. The tumours were minced mechanically in culture medium and the tissue suspensions were filtered through 30 µm filters (Nytal Schweizersche Seidengazefabrik AG). The cell concentration was determined by use of a hemocytometer viewed through a microscope with phase contrast optics. Intact cells which did not take up trypan blue were counted as viable. The cell suspensions were diluted to appropriate concentrations in culture medium.

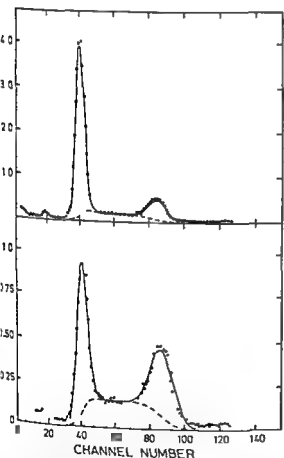
Suspensions of red blood cells (RBC) were prepared from blood of hooded/NOR rats. The serum and the buffy coat were removed after centrifugation. The RBC were rinsed in phosphate buffered saline three times before final resuspension in culture medium to the original blood volume. The RBC suspensions were stored up to three weeks at 4°C. Immediately before use they were diluted 1:1 in culture medium and heated to 44°C for 1 h to destroy remaining nucleated cells.

Agar solutions were prepared by boiling powdered agar (Bacto agar, Difco) with bidistilled water (0.05 g/ml) for 10 min. After being autoclaved at 120°C for 15 min the 5% agar solutions were cooled to 44°C in a water bath and mixed with culture medium at 44°C to make agar solutions of 0.5 per cent. The final soft agar was made by mixing 3 volumes of 0.5% agar solution, 3 volumes of tumour cell suspension and 1 volume of RBC suspension. The temperature of the latter two cell suspensions was 37°C before they were added to the agar solution which had been moved from the water-bath to 44°C to the incubator room at 37°C a few minutes earlier.

Aliquots of 1 ml were immediately seeded in glass tubes. Tubes with ground glass joints and correspondingly coned ground glass stoppers were used. Stopcock grease was applied in the joints to make the tubes gastight. The tubes were plunged into crushed ice after seeding and the soft agar was



1. Mean relative tumour cross-sectional area as a function of time after single dose irradiation of a human malignant melanoma in subcutaneously in nude mice. Vertical bars indicate SD of mean. ● Non irradiated tumours (n=6) ■ Tumours irradiated with 5.0 Gy (n=8) ▲ Tumours irradiated with 10.0 Gy (n=8).



2. DNA histograms analysed by fitting a mathematical model to the experimental data (●). Dashed curves show the mathematical estimate for cells in S. The distribution (in per cent) of cells among the various phases—G₁/G₀ 65, S 20 and G₂+M 15 in non-irradiated tumour (top) and G₁/G₀ 34, S 35 and G₂+M 31 in tumour 4 h after exposure to a single dose of 10.0 Gy (bottom)—obtained from the mathematical analysis. The peaks around channel 19 are due to non parenchymal mouse cells. The channel number is proportional to cellular DNA content.

allowed to set. Immediately afterwards the tubes were flushed with a gas mixture of 5% O₂, 5% CO₂ and 90% N₂. The flushing was repeated about 2 h later. The tubes were incubated at 37°C in vertical position for 3 weeks. Culture medium (2 ml) was added 5 days after seeding and changed 8 days later. The tubes were flushed on each occasion and in addition 3, 9 and 17 days after seeding.

Colonies larger than 40 cells were counted 21 days after seeding. The agar lumps were decanted and carefully squeezed out with a glass-cover on to a specially made sectioned glass plate with a 2 mm high rim. The agar sheets were analysed through a stereo microscope.

Autoradiography. The mice were injected intraperitoneally with a single dose of 1×10^{-6} Ci/g (3.7×10^4 Bq/g) bodyweight (methyl-³H) thymidine (18.8 Ci/mmol, 6.96×10^{11} Bq/mmol) at 8 a.m. At predetermined times the mice were killed and the tumours fixed in 4 per cent formaldehyde. The tumours were then embedded in paraffin wax and sections 3 to 4 μ m thick were cut and mounted on slides. The tissue sections were coated with Ilford L4 nuclear research emulsion by the dipping technique. After an exposure time of 8 weeks at 4°C the slides were developed in Kodak microdol X fixed in Kodafix and stained with hematoxylin and eosin. The mean grain counts over labelled interphase nuclei ranged from 10 to 15. The average background corresponded to less than one grain per cell nucleus and a labelling criterion of minimum three grains per nucleus was adopted. In order to determine the percentage labelled mitoses (PLM) curves 300 mitotic figures from each of the tumours were scored as labelled or not. The labelling indices were determined by analysing 10000 cells from tumours of mice killed 2 h after the thymidine injection.

The PLM-data were analysed by assuming that the time spent by the cells in each of the phases of the cell cycle is described by independent log normal distributions (BARRETT 1966). Theoretical PLM-curves were calculated from different chosen sets of distributions by applying a Monte Carlo program (ROFSTAD et al. 1977a). The set of distributions which gave the best fit to the experimental data was selected by visual inspection.

Results

The mean tumour cross sectional area as a function of time after exposure to single radiation doses

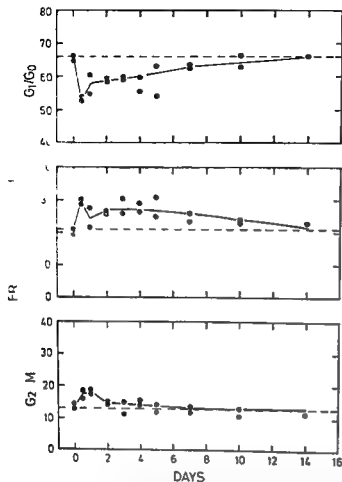


Fig 3 Fraction of cells (in per cent) in G_1/G_0 , S and G_2+M as a function of time after exposure to a single dose of 5.0 Gy for a human malignant melanoma grown in nude mice. Values determined from analysis of DNA histograms. The dashed lines represent the values in non irradiated tumours.

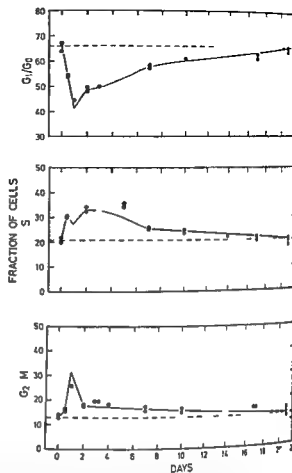


Fig 4 Fraction of cells (in per cent) in G_1/G_0 , S and G_2+M as a function of time after exposure to a single dose of 10.0 Gy for a human malignant melanoma grown in nude mice. Values determined from analysis of DNA histograms. The dashed lines represent the values in non irradiated tumours.

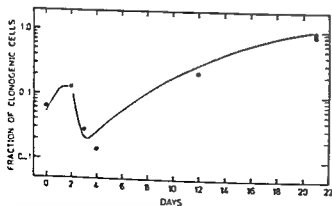


Fig 5 Fraction of clonogenic cells in a human malignant melanoma grown in nude mice as a function of time after exposure to a single dose of 10.0 Gy. Each point represents one tumour. Values calculated in relation to the plating efficiency of cells of untreated tumours of the same size.

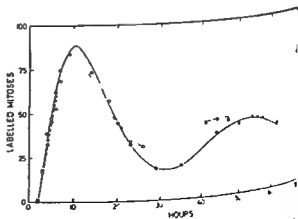


Fig 6 Percentage labelled mitoses curves for a human malignant melanoma grown in nude mice. Non irradiated tumours (O). Tumours dissected free 8 to 11 days after exposure to a single dose of 10.0 Gy (●).

Table 1

Parameters defining the log normal distributions describing the phase durations of the cells of E. E. malignant melanoma grown in nude mice

Phase	Controls tumours			Irradiated tumours		
	Median	Mean	SD	Median	Mean	SD
G ₁	19.0	21.4	11.0	17.7	14.9	9.1
S	13.3	15.1	8.1	17.5	15.3	10.8
G ₂ +M	5.0	5.7	3.0	5.3	6.1	3.5
T	10	10	0.3	10	10	0.3
Labeling index	41.1	43.7	14.0	34.7	37.3	14.6

³H Tdr was administered to the mice 8 days after the tumours were exposed to a single radiation dose of 10.0 Gy.

The distribution for the total cell cycle time T is assumed to be a log normal distribution with mean $m = \sum m_i$ and $SD = \sqrt{\sum \sigma_i^2}$ where m_i and σ_i are the mean and the SD of the four phases into which the cell cycle is divided. The median of this distribution is

$$M = \frac{m}{\sqrt{1 + \frac{\sigma^2}{m^2}}}$$

of 5.0 and 10.0 Gy appears in Fig. 1. The tumour cross sectional areas immediately before irradiation were normalized to unity. The tumour growth was only slightly delayed after 5.0 Gy. The mean tumour cross sectional area and hence the mean tumour volume increased beyond day 8 after exposure to 10.0 Gy but the increase was not as fast as for non irradiated tumours of the same size.

A DNA histogram representative for non irradiated tumours is illustrated in Fig. 2 (upper part). The fraction of the cells in G₁/G₀, S and G₂+M as indicated in the figure legend was calculated by assuming normal distributions for the G₁/G₀ and the (G₂+M)-peaks (DEAN & JETT 1974) while the S fraction (the area under the dashed curve) was modelled as a Gauss broadened exponential function (LINDVIO & AARNES 1979). From the analyses of the DNA histograms of 10 different non irradiated tumours the mean fraction of cells in G₁/G₀, S and G₂+M was found to be 66±2%, 21±2% and 13±2% respectively. Fig. 2 (lower part) shows an example of a DNA histogram of an irradiated tumour (10.0 Gy) where the cell cycle distribution is highly perturbed. The fraction of cells in G₁/G₀, S and G₂+M is 34%, 35% and 31% respectively. The fraction of doublets in cell suspensions from both non irradiated and irradiated tumours was

determined to be 5 to 11 per cent by counting the number of singlets and doublets within a defined area of a hemocytometer by use of a fluorescence microscope. Thus the fraction of cells in G₁+M is somewhat overestimated and the fraction of cells in G₁/G₀ and S correspondingly underestimated in the present analysis.

The distribution of cells among G₁/G₀, S and G₂+M was determined as a function of time after exposure to single doses of 5.0 (Fig. 3) and 10.0 Gy (Fig. 4). The G₁/G₀ fraction decreased considerably during the first 12 h after 5.0 Gy. During the following days the G₁/G₀ fraction increased slowly and reached the level in non irradiated control tumours about 2 weeks after exposure. Both the S and the (G₂+M) fractions have a maximum during the first 2 days after irradiation followed by a slow decrease corresponding to the increase in the G₁/G₀ fraction. Similar curves were obtained after 10.0 Gy (Fig. 4). However the perturbations were more marked and about 3 weeks elapsed until control levels were reached.

In order to facilitate the evaluation of the flow cytometric measurements, more detailed analysis of the proliferation kinetics in tumours exposed to 10.0 Gy was performed. Fraction of clonogenic cells as a function of time after irradiation appears in Fig. 5. Each point represents the value for one tumour calculated in relation to the plating efficiency of cells from non irradiated control tumours of the same size. The plating efficiency was 20 to 30 per cent. Immediately after irradiation the fraction of clonogenic cells was about 0.05. The control level was not reached until 18 to 21 days after irradiation.

The phase durations of the cells of non irradiated tumours and tumours exposed to 10.0 Gy were determined by the PLM technique. ³H Tdr was administered to mice with irradiated tumours 8 days after irradiation. At this time the tumours were regrowing actively as indicated in Figs. 1 and 5. The PLM curves are presented in Fig. 6. The parameters defining the corresponding log normal distributions are summarized in Table 1. The labelling indices were found to be 0.190 for non irradiated tumours and 0.273 for irradiated tumours. By use of the mathematics described by STEEL (1968), growth fraction, potential doubling time and cell loss factor were calculated from the volume doubling time, the median phase durations and the labelling indices (Table 2). It may be concluded that the durations of

Table 2
Cell kinetic parameters for E. E. malignant melanoma grown in nude mice

	Measured values						Calculated values		
	T _D (days)	Median duration (h)					L I	T _{pot} (h)	G F ϕ
		G	S	G ₂	M	T			
Control tumours	7	19.0	13.3	5.0	1.0	41.1	0.190	56	0.66 0.67
Irradiated tumours	14	12.7	12.5	5.3	1.0	34.7	0.273	39	0.85 0.88

T_D = volume doubling time of tumours of the present size determined from growth curves L I = labelling index
T_{pot} = potential doubling time G F = growth fraction ϕ = cell loss factor T_{pot}, G F and ϕ were calculated from the following equations

$$\ln(1 + G F) = \frac{T \ln 2}{T_{\text{pot}}} \quad L I = \exp \left[\frac{\ln 2}{T_{\text{pot}}} (T_G + T) \right] - \exp \left[\frac{\ln 2}{T_{\text{pot}}} T_G \right] \quad \phi = 1 - \frac{T_{\text{pot}}}{T_D}$$

(Steel 1968)

³H Tdr was administered to the mice 8 days after the tumours were exposed to a single radiation dose of 10.0 Gy

G₂ and S are not significantly different in irradiated and non irradiated tumours while G₁ is shortened in irradiated tumours. The growth fraction and the cell loss factor are considerably larger in irradiated tumours than in non irradiated tumours.

Discussion

A previous investigation has indicated that the phase durations of the cells of human tumours grown in nude mice are in the range of those found for tumour cells in man (ROFSTAD *et al.* 1977a). The phase durations of the cells of non irradiated E. E. malignant melanoma are in agreement with this conclusion.

The labelling index of non irradiated tumours (0.190) indicates that all cells in the S fraction as measured by flow cytofluorometry (0.21) are synthesizing DNA, i.e. it is not likely that a significant proportion of the cells are arrested in S. As the growth fraction was found to be 0.66 (66%) and the (G + M) fraction contributes about 13 per cent of the population, most of the non proliferating cells (34%) must have the same DNA content as proliferating cells in G₁. A corresponding analysis of the data obtained for irradiated tumours (Table 2, Fig. 4) leads to the same conclusion. Similar results were previously obtained for untreated NHIK 1922 cells grown in nude mice (ROFSTAD *et al.* 1980).

Figs 2, 3 and 4 show that tumour cells are accumulated in G + M during the first day after irradiation. This accumulation is to be expected since

radiation induced mitotic delay is well known from a considerable number of investigations, both in vitro and of solid tumours. By assuming that the flux of cells to G₁/G₀ is negligible during the first 24 h after the tumours are exposed to a high radiation dose, and since the median duration of G₁ in non irradiated tumours is 19.0 h, it follows that the G₁/G₀ peak of the DNA histograms observed 24 h after the exposure may be ascribed mainly to non proliferating cells or slowly proliferating G₂ cells. The fraction of cells in the G₁/G₀ peak 24 h after exposure to 10.0 (ca. 40% Fig. 4) and 20.0 Gy (ca. 35% Fig. 2) corresponds fairly well with the fraction of non proliferating cells in non irradiated tumours (34%). Thus, also the DNA histograms obtained 24 h after exposure to 10.0 and 20.0 Gy indicate that most of the non proliferating cells in the tumour have the same DNA content as proliferating cells in G₁.

The DNA histograms obtained with the present technique include radiation inactivated cells which are morphologically intact. The fraction of clonogenic cells was about 5 per cent immediately after exposure to 10.0 Gy and not until 4 days later did this fraction start to increase towards the value in non irradiated tumours (Fig. 5). The DNA histograms from the first day after irradiation were therefore mainly based on cells lethally damaged by radiation. The radiation inactivated cells became less important with time after irradiation as they disintegrated and were removed.

The present experiments may explain some of the

mechanisms underlying the gross radiation response of E E malignant melanoma. Even though about 5 per cent of the cells were inactivated after 10.0 Gy (Fig. 5) the tumour volume after irradiation never decreased below its value at irradiation (Fig. 1). The tumour volume continued to increase for about 4 days after exposure, indicating that inactivated cells may have the ability to divide a few times before being lysed. As the S and (G_1+M) fractions increased and the G_1/G_0 fraction decreased (Fig. 4) the average volume per cell increased. In addition the tumour volume may have increased because of radiation induced edema. The following 4-day period was characterized by a moderate tumour shrinkage, indicating that removal of inactivated cells now dominated. Beyond day 8 after irradiation the rate of production of new cells was higher than the rate of removal of inactivated cells, resulting in an increase in tumour volume.

The fraction of clonogenic cells increased by a factor of 2 to 3 during the first day after exposure to 10.0 Gy (Fig. 5). As the cell cycle time in this period was especially long due to mitotic delay, this increase was probably not due to proliferation of surviving cells. Neither is destruction of inactivated cells a probable explanation since the tumour volume continued to increase after irradiation. The effect appears rather to be a consequence of repair of potentially lethal damage (HAHN *et al.* 1974, SHIPLEY *et al.* 1975). From day 2 to day 3 to 4 after irradiation the fraction of clonogenic cells decreased considerably. There is evidence that cell death due to anoxia, nutrient deficiency or both conditions occurs in poorly vascularized tumours (THOMLINSON 1960, TANNOCK 1972, YAMAURA & MATSUZAWA 1979). One possible explanation for the present decrease may be that a large fraction (80-90%) of the surviving radiation resistant hypoxic cells were inactivated 3 to 4 days after irradiation, not because of radiation injury to the cells themselves but rather because of anoxia, nutrient deficiency or both. Corresponding curves for the rat rhabdomyosarcoma R 1 (HERMENS & BARENDSEN 1969) and the rat fibrosarcoma RIB₃C (MCNALLY 1973) did not show a fall similar to that observed here. It may be speculated that for tumours with shorter cell cycle time than the present one the effects of repair of potentially lethal damage and inactivation due to anoxia, nutrient deficiency or both may cancel each other. However, when the central part of the rhabdomyosarcoma R 1 which

contains mainly radiation resistant hypoxic cells was analysed separately, the fraction of clonogenic cells was found to be significantly lower 2 days after irradiation as compared to immediately afterwards (HERMENS & BARENDSEN 1975). The fraction of clonogenic cells in E E malignant melanoma increased beyond the fourth day after irradiation (Fig. 5). This may be due to both proliferation of surviving cells and destruction and removal of inactivated cells. As the tumour volume decreased 4 to 8 days after irradiation (Fig. 1) the last factor was probably the most important one in this time interval, while the first factor probably dominated beyond day 8 when the tumour volume increased.

The PLM investigation (Fig. 6) was carried out 8 to 11 days after irradiation. Figs 1 and 5 show that the tumours were regrowing actively at the time. The cell cycle time in irradiated tumours was shorter than that in non irradiated tumours due to a shorter G_1 . In agreement with the present results the cell cycle time in the rat rhabdomyosarcoma R 1 4 to 14 days after exposure to 20.0 Gy was found to be considerably shortened, mainly because of disappearance of G_1 (HERMENS & BARENDSEN 1969, 1970, 1975). VAN PEPERZEEL observed a reduced duration of all phases of the cell cycle 45 h after pulmonary metastases of the murine adenocarcinoma M 8013 were exposed to 2.1 Gy. On the other hand a slight prolongation of G_1 was observed in a squamous cell carcinoma of the hamster cheek pouch within 4 days after exposure to 5.0 and 10.0 Gy (BROWN & BERRY BROWN). Similarly most of the PLM-curves obtained for the murine adenocarcinoma 284 in the interval 1 to 8 days after doses of 6.0 and 12.0 Gy indicated a prolongation of the cell cycle time, mainly G_1 (SZCZEPANSKI & TROTT).

In addition to reduced duration of G_1 , the growth fraction in E E malignant melanoma was increased after irradiation (Table 2). Increased growth fraction was also observed in the adenocarcinomas M 8013 (VAN PEPERZEEL) and 284 (SZCZEPANSKI & TROTT) and the fibrosarcoma NCTC 2472 (MALAISE & TUBIANA 1966, TUBIANA *et al.*) while the growth fraction in the rhabdomyosarcoma R 1 was slightly reduced (HERMENS & BARENDSEN 1969, 1970, 1975).

As the cell cycle time was reduced and the growth fraction increased in E E malignant melanoma it may be concluded that the rate at which new cells were produced was higher in irradiated tumours during regrowth than in non irradiated tumours of

the same size. In spite of this the volume of the irradiated tumours increased more slowly than that of the non irradiated tumours (Fig. 1) due to more extensive cell loss (Table 2). Similar regrowth curves have been reported for instance for the fibrosarcoma RIB₃ (THOMLINSON & CRADDOCK 1967) and the rhabdomyosarcoma R 1 (BARENDSEN & BROERSE 1969) but also increased overall growth rate after irradiation has been demonstrated (VAN PEPERZEEL). The latter author reported the cell loss factor to be reduced after irradiation.

As the majority of the non proliferating cells have the same DNA content as proliferating cells in G₁, reduced duration of G₁ and increased growth fraction should result in reduced G₁/G₀ fraction and increased S and (G + M) fractions. The flow cytometric measurements in Fig. 4 are consistent with the ³H Tdr incorporation data in Table 2. Thus Fig. 4 indicates that the combined effect of a reduced G₁ and an increased growth fraction and hence the rate at which new cells were produced was largest during the first days after the cells were released from the G₂ block. The G₁/G₀ fraction increased and the S and (G + M) fractions decreased over the next days and about 3 weeks after exposure the distribution of cells among G₁/G₀, S and G + M was the same as in non irradiated tumours. Even 50 Gy a dose which caused minor changes in the overall tumour growth (Fig. 1) affected the proliferation kinetics in the same manner as the higher dose of 100 Gy. However the effect was less marked and ceased about 2 weeks after exposure. The present results are supported by those reported by DENEKAMP & THOMLINSON. In 4 animal tumours but at times corresponding to at least 10 cell cycles after doses of 10.0 and 15.0 Gy they did not observe significant changes neither in the phase durations nor in the growth fractions.

SUMMARY

The effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma grown in the athymic mutant nude mouse was analysed. DNA histograms were obtained with flow cytofluorometry. Percentage labelled mitoses curves were established by the use of conventional autoradiographic techniques. Changes in the fraction of clonogenic cells with time after irradiation were measured in vitro in soft agar. In non irradiated tumours the fraction of cells in G₁/G₀, S and G₂+M was 66, 21 and 13 per cent respectively. The median duration of G₁, S, G₂, M and T was 19.0 h, 13.3 h, 5.0 h, 1.0 h and 41 h respectively. The growth fraction was calculated as 0.66

and the cell loss factor as 0.67. The growth fraction increased after irradiation and the cell cycle time reduced due to a shortening of G₁. These effects were dose dependent and decreased with time after exposure but were present after the tumours had resumed a continuous volume growth. The rate of volume growth was slower for irradiated tumours than for non irradiated tumours the same size due to a larger cell loss factor for the former.

ACKNOWLEDGEMENTS

The authors are grateful to the director of the Department of Surgery, The Norwegian Radium Hospital Dr O. Brennhovd for supplying the human melanoma tissue and to K. Fundingsrud for operating the flow cytometer. Financial support from the Norwegian Cancer Society, The Norwegian Research Council Science and the Humanities and the Nansen Science Fund is acknowledged.

REFERENCES

- BARENDSEN G. W. and BROERSE J. J. Experimental radiotherapy of a rat rhabdomyosarcoma with 15 kV neutrons and 300 kV X rays. I. Effects of single exposures. *Europ. J. Cancer* 5 (1969) 373.
- BARRETT J. C. A mathematical model of the mitotic cycle and its application to the interpretation of percent labelled mitoses data. *J. Nat. Cancer Inst.* 37 (1969) 443.
- BRAUNSCHWEIGER P. G., SCHENKEN L. L. and SCHULTZ L. M. The cytokinetic basis for the design of efficacious radiotherapy protocols. *Int. J. Radiat. Oncol. Biol. Phys.* 5 (1979) 37.
- BROWN J. M. The effect of acute X irradiation on the proliferation kinetics of induced carcinomas and the normal counterpart. *Radiat. Res.* 43 (1970) 697.
- and BERRY R. J. Effects of X irradiation on the population kinetics in a model tumour and normal tissue system. Implications for the treatment of human malignancies. *Brit. J. Radiol.* 42 (1969) 379.
- CAIRNIE A. B. Cell proliferation studies in the intestinal epithelium of the rat. Response to continuous irradiation. *Radiat. Res.* 32 (1967) 240.
- COURTFENAY V. D. A soft agar colony assay for lung tumour and B16 melanoma taken directly from mouse. *Brit. J. Cancer* 34 (1976) 39.
- and MILLS J. An in vitro colony assay for human tumours grown in immune suppressed mice treated in vivo with cytotoxic agents. *Brit. J. Cancer* (1978) 261.
- CRISMAN H. A. and TORREY E. A. Cell cycle analysis. 20 minutes. *Science* 184 (1974) 1297.
- DEAN P. N. and JETT J. H. Mathematical analysis of DNA distributions derived from flow microfluorimetry. *J. Cell Biol.* 60 (1974) 523.
- DENEKAMP J. and THOMLINSON R. H. The cell proliferation kinetics of four experimental tumours after X irradiation. *Cancer Res.* 31 (1971) 1799.

- 1 BALL M M and FOWLER J F Recovery and repopulation in mouse skin as a function of time after X irradiation *Radiat Res* 37 (1969) 361
- 2 UNDEL E VASSORT F and TUBIANA M Effects of irradiation on the cell cycle of an experimental ascites tumour of the mouse *Int J Radiat Biol* 17 (1970) 39
- 3 ROCKWELL S KALLMAN R F GORDON L F and FRINDEL E Repair of potentially lethal damage in vivo in solid tumor cells after X irradiation *Cancer Res* 34 (1974) 351
- 4 BARENDSEN G W Changes of cell proliferation characteristics in a rat rhabdomyosarcoma before and after X irradiation *Europ J Cancer* 5 (1969) 173
- 5 Proliferation characteristics of cells in an experimental rhabdomyosarcoma at various time intervals before and after irradiation *In Proceedings of Conference on Time and Dose Relationships in Radiation Biology as Applied to Radiotherapy* p 147 Brookhaven National Laboratory Upton BNL 50203 (C 57) 1970
- 6 The importance of proliferation kinetics and clonogenicity of tumor cells for volume responses of experimental tumors after irradiation *In Radiation research biomedical chemical and physical perspectives* p 834 Edited by O F Nygaard H I Adler and W K Sinclair Academic Press New York 1975
- 7 SILINIG F and TAYLOR H M Recupération from lethal injury by whole body irradiation. II Kinetic aspects in radiosensitive BALB/c mice and cyclic time structure during the four days after conditioning irradiation *Radiat Res* 29 (1966) 362
- 8 HOPKINS H A EVANS M J and LOONEY B Changes in cellularity induced by radiation in a solid tumour *Int J Radiat Biol* 30 (1976) 101
- 9 Compensatory reactions in intestinal crypt cells after 300 roentgens of cobalt 60 gamma irradiation *Radiat Res* 32 (1967) 510
- 10 T and AARNES E Selection of optimal model for the DNA histogram by analysis of error of estimated parameters *J Histochem Cytochem* 27 (1979) 797
- 11 PETERSEN E O Delay of cell cycle progression after X irradiation of synchronized populations of human cells (NHK 3025) in culture *Cell Tissue Kinet* 1 (1979) 43
- 12 STEEN H B Flow cytometric measurement of the polarization of fluorescence from intracellular fluorescent in mammalian cells *Biophys J* 18 (1977) 173
- 13 TUBIANA M Croissance des cellules d'un tumeur sous irradiation expérimentale chez la souris C3H *C R Acad Sci* 263D (1966) 292
- 14 A comparison of the effects of radiation on tumour growth delay and cell survival. The effect of oxygen *Brit J Radiol* 46 (1973) 450
- 15 S R CARPENTER R E and DURBORAW M Mechanisms underlying reduced growth rate in C3H4 mammary adenocarcinomas recurring after single doses of X rays or fast neutrons *Cancer Res* 36 (1976) 574
- 16 COBB L M and STEEL G G The growth kinetics of xenografts of human colorectal tumours in immune deprived mice *Brit J Cancer* 31 (1975) 36
- 17 ROCKWELL S and KALLMAN R F Cyclic radiation induced variations in cellular radiosensitivity in a mouse mammary tumour *Radiat Res* 57 (1974) 132
- 18 ROSTAD E K BRISTAD T and KAAHUS O (a) Cell proliferation kinetics in two human tumors grown in athymic nude mice *Virchows Arch B Cell Path* 24 (1977) 219
- 19 JOHANNESSEN J V and MOSSIGE J (b) Effect of cobalt 60 gamma rays and DTIC (5 (3,3 dimethyl 1 trizeno) imidazole 4 carboxamide) on human malignant melanomas grown in athymic nude mice *Brit J Radiol* 50 (1977) 14
- 20 PETERSEN E O LINDEG T and OFTEBRO M The proliferation kinetic of NHK 922 cells in vitro and in solid tumours in athymic mice *Cell Tissue Kinet* 13 (1980) 163
- 21 The use of metrizamide for the separation of rat liver cells *In Biological separations in iodinated density gradient media* p 107 Edited by D Rickwood Information Retrieval Limited London and Washington DC 1976
- 22 SHIPLEY W U STANLEY J A COURTENAY V D and FIELD S P Repair of radiation damage in Lewis lung carcinoma cells following in situ treatment with fast neutrons and gamma rays *Cancer Res* 35 (1975) 932
- 23 STEEL G G Cell loss from experimental tumours *Cell Tissue Kinet* 1 (1968) 193
- 24 Growth kinetics of tumours. Cell population kinetics in relation to the growth and treatment of cancer Clarendon Press Oxford 1977
- 25 SZCZEPANSKI L v and TROTT K E Post irradiation proliferation kinetics of a serially transplanted murine adenocarcinoma *Brit J Radiol* 48 (1975) 200
- 26 TANNOCK I F Oxygen diffusion and the distribution of cellular radiosensitivities in tumours *Brit J Radiol* 45 (1972) 515
- 27 THOMLINSON R H An experimental method for comparing treatments of intact malignant tumours in animals and its application to the use of oxygen in radiotherapy *Brit J Cancer* 14 (1960) 553
- 28 and CRADDOCK E A The gross response of an experimental tumour to single doses of X rays *Brit J Cancer* 21 (1967) 108
- 29 TUBIANA M The kinetics of tumour cell proliferation and radiotherapy *Brit J Radiol* 44 (1971) 325
- 30 FRINDEL E and MALAISE E The application of radiobiologic knowledge and cellular kinetics to radiation therapy *Amer J Roentgenol* 102 (1968) 872
- 31 VAN PEPERZEEL H A Effects of single doses of radiation on lung metastases in man and experimental animals *Europ J Cancer* 11 (1972) 665
- 32 WITHERS H R and ELKIND M M Radiosensitivity and fractionation response of crypt cells of mouse jejunum *Radiat Res* 38 (1969) 598
- 33 YAMAMURA H and MATSUZAWA T Tumour regrowth after irradiation An experimental approach *Int J Radiat Biol* 35 (1979) 201

DOSE EFFECT RELATIONSHIPS IN CERVICAL AND THORACIC RADIATION MYELOPATHIES

B. HOLDORFF

In 1968 ZEMAN reported that the latency period for myeloses following a single dose of irradiation was strictly time dependent. Since then this question has been repeatedly discussed in connection with radiation myelopathies in man. Current experience implies that no clear negative relationship between the dose and latency period after fractionated radiation exists (FRANKE 1973). This also holds true for animal experiments in which a latency period of four to five months cannot be further reduced under fractionated radiation (VAN DER KOOG & BARENDSEN 1974).

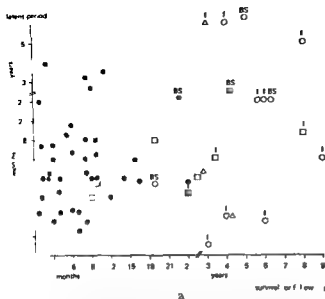
On the other hand radiation myelopathies have often been reported that prove fatal after a short latency period and a progressive course versus other conditions with a long interval and a long survival time. These suggest that the latency period and the extent of the lesions in the survival time may depend upon the irradiation doses (latency period—survival time—relationship). Thus HUNG (1968) found an average latency period of 18 months among the fatal cases of his series versus an average of 25.4 months latency in contrast to ATKINS & TRETTER (1966) as well as JELLINGER & STURM (1971) who failed to detect any correlation between the latency period, the severity of clinical symptoms and signs and survival time or any dependence of these factors on the radiation dose. This in turn does not corroborate the result of GANSHIRT (1978). In a relatively homogeneous group of patients with complications subsequent to radiation therapy of the lymphatic

system he found a rough relationship between the dose and the severity of neurologic syndromes. Evidently the question of dose effect relationships remains a matter of considerable controversy.

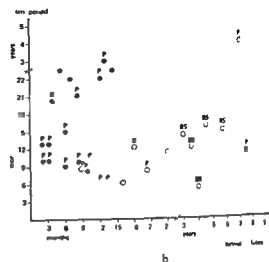
Material and Methods

The present series and well-documented cases in the literature were entered into a latency catamnesis diagram to describe the course types (Figure) and classified according to cervical and thoracic radiation myelopathies. Lumbar or thoracolumbar myelopathies were excluded from the series because they are rare and often incorrectly classified as to the exact localisation, the so-called amyotrophic type being not a myelopathy at all but rather a peripheral neuropathy (HOLDORFF 1979). Only those cases in the literature were considered that provided a precise and extensive documentation of the neurologic status and the course of the patients.

The cases of radiation therapy of the lymphatic system and myelopathies following two radiation cycles were treated separately. The observation after irradiation of the lymph nodes was classified together with the cervical radiation myelopathies as the classification was based on neurologic findings, autopsy results not always being available. Occasionally thoracically located spinal necroses could not be entirely ruled out.



Radiation myelopathies a) Cervical b) Thoracic Living (O) deceased (●) Living (□) deceased (■) after 2 series of irradiation Living (Δ) deceased (▲) after mantle field irradiation



Paraplegia (P) Brown Séquard (BS) Incomplete transverse syndrome (I)

Due to the large field of irradiation the question of the exact location of the necrosis remains open. The neurologic status indicates a level which does not necessarily correspond to the level of the spinal necroses. These may at times extend higher into the cervical region even though neurologic signs indicate the thoracic region of the spinal cord (CASTAIGNE *et coll.* 1970).

These difficulties of the localization do not as a rule occur in those radiation myelopathies following a small irradiated volume because in these cases irradiation field and spinal cord lesions coincide.

The appearance of the first neurologic symptoms of radiation myelopathy marked the beginning of the survival time or follow-up period. The terminal stages of the neurologic disease were designated by letters; this was not possible in cases of cervical radiation myelopathies with a fatal outcome for which frequently no documentation of the neurologic findings at the terminal stage was available but only a status taken days or weeks before the final phase. One can only speculate that in the final phase transverse syndromes with partial involvement of the upper extremities developed into upper tetraplegic transverse syndromes. Paraplegias in cases of thoracic radiation myelopathies were on the other hand documented reliably.

Though the relationship between the latency period and the survival or follow-up period and in turn their dependence upon the radiation dose were of primary interest. The extent to which various

neurologic criteria and the segmental localization of the spinal cord lesion influenced course and prognosis was also analyzed.

Results

The Figure does not allow a generalization and postulation of a linear relationship between the latency period and the survival time though such a relationship might be considered for some of the thoracic paraplegia cases. On the other hand it is crucial for the clinical course whether the radiation myelopathy progresses to a complete transverse syndrome i.e. tetraplegia due to cervical lesions and to paraplegia because of thoracic ones. The final outcome results in the majority of such cases within 10 to 18 months after the appearance of the first symptoms (black symbols in the Figure). The comparison of the latency periods of those patients who died early with those who died later—after 18 months—and with the survivors yields no statistically significant difference in the Wilcoxon test. This applies to both the cervical and the thoracic radiation myelopathies. Only those cases were considered in which the possible cause of death was radiation myelopathy or a secondary effect such as respiratory insufficiency with pneumonia, hypoxia or pulmonary embolism. The surviving patients present mainly with incomplete spinal syndromes either as Brown Séquard syndromes or as incomplete transverse syndromes, these allowing a more

Table 1

Nominal standard dose in cervical radiation myelopathies

Fatal course			Non fatal course		
NSD	Authors	Case No	NSD	Authors	Case No
14.7	SEITZ & KALM (1961)	3	11.3	ZEMAN & SHIDNJA (1976)	
14.7	MARTY & MINCKLER (1973)		11.3	JELLINGER & STURM (1971)	10
14.3	JELLINGER & STURM (1971)	12	12.9	GLANZMAN et coll (1976)	5
14.3	PALMER (1977)	7	14.6	JELLINGER & STURM (1971)	7
14.8	PALMER (1977)	2			
17.7	JELLINGER & STURM (1971)	3	15.5	SOLHEIM (1971)	3
18.1	LECHEVALIER et coll (1973)	5	17.3	SOLHEIM (1971)	4
18.1	LECHEVALIER et coll (1973)	1	20.8	SOLHEIM (1971)	2
			Median 14.6		
18.6	JELLINGER & STURM (1971)	8			
19.1	JELLINGER & STURM (1971)	4			
19.8	HOLDORFF (1979)	1			
20.1	JELLINGER & STURM (1971)	9			
21.9	JELLINGER & STURM (1971)	2			
27.5	JELLINGER & STURM (1971)	11			
23.8	LECHEVALIER et coll (1973)	2			
Median 18.1					

favorable prognosis quoad vitam. Yet both short and long latency periods occur and the series allows no correlation between the latency period and dose or other modalities of irradiation such as two radiation series within a few months and high radiation volumes in cases of radiation therapy of the lymphatic system.

No significant difference was found in the prognosis between lesions of the upper and lower cervical segments.

For 36 fatal cervical radiation myelopathies a median survival time of 6.5 months is calculated ranging from 2 to 16.5 months and for 18 fatal thoracic radiation myelopathies a median survival time of 7.5 months ranging from 2 to 15 months is found. For the six fatal cases following two consecutive irradiation cycles performed within a few months the median survival time lay at 3 (2-10) months and is particularly short.

Under the hypothesis that fatal and non fatal courses or complete and incomplete transverse syndromes were the result of different radiation doses a possible dose effect relationship was sought. Only those cases could be referred to in which the nominal standard dose (NSD) was either specified or could be calculated. The NSD value was calculated according to the formula of Ellis

$$NSD = D \times N^{-0.24} \times T^{-0.11}$$

As the dose D was expressed in Gy the NSD value was expressed in 100 ret. If the median spinal dose is calculated for those patients who died within the first 18 months and for the group of survivors (Table 1) NSD of 18.1 (median value) is yielded for the 15 fatal cervical cases and a NSD of 14.6 for the 7 survivors with incomplete transverse syndromes. The difference is significant at the 10 per cent level.

Almost identical NSD values of about 15.4 are calculated for the fatal and non fatal thoracic cases. However if the complete and incomplete thoracic transverse syndromes are separated (Table 2) a NSD of 16.9 is found in the first group of 14 cases and a NSD of 15.3 for the second group of 11 cases. This difference is statistically significant at the 5 per cent level of the Wilcoxon test. For radiation myelopathies following irradiation of the lymphatic system (Table 3) a median NSD value of 15.2 is calculated for complete transverse syndromes in 4 cases and a NSD value of 13.2 for 5 incomplete transverse syndromes. The difference is again significant at the 5 per cent level.

If incomplete transverse syndromes following upper mantle field irradiation (Table 3) are compared with incomplete thoracic transverse syndromes

Table 2
Nominal standard dose in thoracic radiation myelopathies

Complete transverse syndrome			Incomplete transverse syndrome		
NSD	Authors	Case No	NSD	Authors	Case No
14.5	LOCKSMITH & POWERS (1968)	1	14.5	LOCKSMITH & POWERS (1968)	1
15	PALMER (1972)	5	14.5	LOCKSMITH & POWERS (1968)	4
15	PALMER (1972)	6	15	LOCKSMITH & POWERS (1968)	5
15.3	PHILLIPS & BUSCHKE (1969)	1			
16.1	PALMER (1972)	1	15.5	PALMER (1972)	3
16.7	LOCKSMITH & POWERS (1968)	3	16	GLANZMANN et coll (1976)	3
16.7	LOCKSMITH & POWERS (1968)	6	16.1	ATKINS & TRETTER (1966)	11
17	ATKINS & TRETTER (1966)	12	Median 15.3		
17.5	GLANZMANN et coll (1976)	7			
17.6	COY et coll (1969)	1			
17.9	COY et coll (1969)	2			
18.1	COY et coll (1969)	3			
19.7	PHILLIPS & BUSCHKE (1969)	3			
19.9	PHILLIPS & BUSCHKE (1969)	2			
Median 16.9					

Table 3
Nominal standard dose in radiation myelopathies after upper mantle field irradiation

Complete transverse syndrome			Incomplete transverse syndrome		
NSD	Authors	Case No	NSD	Authors	Case No
13.4	GLANZMANN et coll (1976)	5	12.5	GANSHIRT (1975)	1
14.9	GANSHIRT (1975)	6	13	GLANZMANN et coll (1976)	4
15.4	GANSHIRT (1975)	9	13.2	GANSHIRT (1975)	4
16.7	GANSHIRT (1975)	3	13.4	HOLDORFF (1979)	1
			13.7	GANSHIRT (1975)	7
Median 15.2			Median 13.2		

(Table 2) ■ median NSD value of 13.2 and 15.3 respectively is found. These values differ significantly at the 5 per cent level. Despite the small number of cases in both groups the statistical comparison is justifiable because all NSD values in the group following upper mantle field irradiation are smaller than the smallest value of the other group with thoracic radiation myelopathies. A part of the results is compiled in Table 4.

Discussion

The present results indicate that the fatal outcome of many cervical and thoracic radiation mye-

lopathies occurs within 18 months of the appearance of the first neurologic symptoms i.e. when they develop into a complete transverse syndrome while incomplete transverse syndromes are consistent with a longer survival time.

An attempt to distinguish upper and lower cervical radiation myelopathies by morphologic and neurologic criteria does not yield any clinically useful differences in the course of the ultimate fatal cases. The median survival time of 6.4 months for fatal cervical myelopathies does not essentially differ either from the median survival time of 7.4 months for 18 fatal thoracic myelopathies. Nevertheless for the course and the prognosis

Table 4

Nominal standard dose in radiation myelopathies

Cervical		Thoracic		After upper mantle field irradiation	
Fatal	18.1	Complete	16.9	Complete	15.2
Non fatal	14.6	Incomplete	15.3	Incomplete	13.2
$2\alpha < 10^{-6}$		$2\alpha < 5^{-6}$		$2\alpha < 5^{-6}$	

portance of the segmental localization of spinal crosses should not be ignored

It is known that in cases of traumatic transverse lesions the mortality rate depends upon the segmental localization of the spinal lesion and increases particularly for lesions in the upper cervical part of the medulla i.e. C1-C4 mainly due to acute respiratory paralysis (MESARD et coll 1978). In the cases of cervical radiation myelopathy it is difficult to distinguish different courses of upper and lower cervical radiation myelopathy because of the inhomogeneous distribution of the necroses over a large segment of the spinal cord

Furthermore the present analysis proceeded on the assumption that the dose causing spinal cord necrosis would have to differ for complete and incomplete transverse syndromes. This assumption was confirmed statistically for those cases with a known NSD. The median spinal necrosis dose mentioned is not identical with the threshold of tolerance which lies well below the range of necrosis doses. Nevertheless the spinal cord necrosis dose of incomplete radiogenic transverse syndromes comes closest to the threshold tolerance because it represents a submaximum overdosage and not a maximum one as in the case of complete transverse syndromes. The scattering range of NSD is as expected small (Tables 2-3). For this reason incomplete transverse syndromes are best suited for determining the threshold of tolerance. This is only possible when the clinical course may be observed for a sufficiently long period of time so that a later progression to a complete transverse syndrome may be excluded. According to this principle a median spinal necrosis dose of NSD 15.3 and 13.2 could be determined for the incomplete thoracic radiation myelopathies and for those following lymph node irradiation respectively.

This division into complete and incomplete transverse syndromes cannot be applied to cases of cer-

vical radiation myelopathy because in many cases of the literature incomplete cervical transverse syndromes were described with an early fatal outcome where presumably the complete tetraplegic transverse syndrome in the final phase was not recorded. On the other hand all surviving patients presented with an incomplete transverse syndrome either unilaterally along the lines of the Brown Sequard syndrome or as a bilateral incomplete transverse syndrome. Therefore it is suggested for cervical radiation myelopathies that the course criterion fatal corresponds to complete and non fatal to incomplete transverse syndromes. A mean spinal necrosis dose of NSD 14.6 was determined for non fatal incomplete cervical radiation myelopathies.

These criteria for neurologic differentiation have up to now not been applied in the reports on radiation myelopathy risk. According to compilations of data the critical range in which radiation myelopathies may occur fluctuates between NSD values of 10 and 20 (FRANKE) but most often lies above 15 (PHILLIPS & BUSCHKE 1969; GLANZMANN et coll 1976). For reasons of safety a tolerance dose 10 per cent lower than this is applied to the cervical part of the medulla (GLANZMANN et coll). In contrast ABBATUCCI et coll (1978) consider a dose of 50 Gy (25 fractionations in 35 days = NSD 15.7) to be safe if a longitudinal segment of the cervical region of the spinal cord covering no more than three to five vertebrae is irradiated. For the thoracic region of the spinal cord WARA et coll (1975) calculated a 50 per cent incidence rate of approximately NSD 16 while RHEINHOLD et coll (1976) determined one of NSD 19.8 which however is probably far too high. This error may be due to the fact that because of the average latency period of 18 months many cases are not recorded in morbidity investigations of radiation myelopathy because of premature death due to the primary disease (FRANKE).

Tolerance calculations do not fully appreciate the

volume factor although in principle its significance has long been known. As early as 1950 BODEN found that a radiation myelopathy occurred if a longitudinal spinal cord segment of more than 20 cm was irradiated with an otherwise well tolerated dose. ATKINS & TRETTER, ZEMAN & SHIDNIA and AB BATUCCI et coll. also stressed the significance of the volume factor. It is also of importance when considering the craniospinal irradiation of children. The present results emphasize that also an increased risk of radiation myelopathy is involved in high volume irradiation of the lymphatic system in cases of Hodgkin's disease and other forms of malignant neoplastic disease. Incomplete transverse syndromes after a mantle field irradiation of this type (Table 3) and incomplete thoracic radiation myelopathies after low volume irradiation (Table 2) show a significant difference in their median spinal necrosis dose (5 per cent level). This statistical comparison is justifiable in spite of the small number of cases because all NSD values of the group after mantle field irradiation are lower than the lowest values of the other group with thoracic radiation myelopathy. Thus it can be stated that the limit of tolerance for the spinal cord for mantle field irradiations is lower and a safety limit of NSD 12 may be assumed. These results indicate that it would be logical to introduce the volume factor to the conventional NSD formula (GREMMEL & WENDHAUSEN 1977) especially in view of the radiation myelopathy risk.

SUMMARY

The course and prognosis of radiation myelopathies are determined by 3 factors: the segmental (vertical) location of the lesion, the extent of the transverse syndrome (complete or incomplete) and the radiation dose. The median spinal dose in cervical radiation myelopathies with fatal outcome was higher than in survivals with an incomplete transverse syndrome. In thoracic radiation myelopathies a dose difference between complete and incomplete transverse syndromes could be found as well. Incomplete transverse syndromes as submaximum radiation injuries are more suitable for the determination of the spinal tolerance dose than complete transverse syndromes. The lowest threshold could be stated for cases following high volume irradiation of the lymphatic system.

REFERENCES

- ABBATUCCI J S, DELOZIER T, QUINT R, ROUSSEL A and BRUNF D. Radiation myelopathy of the cervical spinal cord. *Time, dose and volume factors*. *Int J Radiat Oncol Biol Phys* 4 (1978) 739.
- ATKINS H L and TRETTER P. Time-dose-considerations in radiation myelopathy. *Acta radiol Ther Phys Biol* 5 (1966) 79.
- BODEN G. Radiation myelitis of the brain stem. *J Fa. Radiol* 2 (1950) 79.
- BURNS H J, JONES A N and ROBERTSON J S. Pathology of radiation myelopathy. *J Neurol Neurosurg Psychiatr* 35 (1972) 888.
- CASTAIGNE P, CAMBIER J, ESCOURDIE R, LECHEVALIER H, TANZER J and LILLIER M. Les myelopathies post radiotherapiques au cours de la maladie de Hodgkin. *Rev neurol* 173 (1970) 769.
- COY P, BAKER S and DOLMAN C L. Progressive myelopathy due to radiation. *Canad med Ass J* 101 (1969) 1129.
- FRANKE H D. Die Strahlenempfindlichkeit des Zentralnervensystems. *In: Deutscher Röntgenkongress 1970*, p. 83. Herausgegeben von O. Hug. Thieme Stuttgart 1973.
- GÄNSHIRT H. Strahlenmyelopathien. *Nervenarzt* 46 (1975) 562.
- Strahlenmyelopathie. *Med Welt* 79 (1978) 761.
- GLANZMANN CH, ABERLE H G and HORST W. Therapy of chronic progressive radiation myelopathy. *Strahlentherapie* 152 (1976) 363.
- GREMMEL H and WENDHAUSEN H. Berücksichtigung der Volumenabhängigkeit von Toleranzdosen. *Strahlentherapie* 153 (1977) 462.
- HOLDORFF B. Klinik der radiogenen Spätschäden des zentralen und peripheren Nervensystems. *Habilitationsschrift Berlin* 1979.
- HUNG T P. Myelopathy following radiotherapy of nasopharyngeal carcinoma. *Proc Austral Asiat Neurol* 5 (1968) 421.
- JELLINGER K and STURM W. Delayed radiation myelopathy in man. Report of 12 necropsy cases. *J Neurol Sci* 14 (1971) 389.
- VAN DER KOEGL A J and BARENDSEN G W. Late effects of spinal cord irradiation with 300 kV X rays and 10 MeV neutrons. *Brit J Radiol* 47 (1974) 393.
- LECHEVALIER H, HUNFAU F and HOUTTEVILLE J P. Myelopathies radiotherapeutiques hypertrophiques. A propos de cinq observations dont une avec syndrome clinique. *Rev neurol (Paris)* 129 (1973) 119.
- LOCKSMITH J P and POWERS W E. Permanent radiation myelopathy. *Amer J Roentgenol* 107 (1969) 916.
- MARTY H and MINCKLER D. Radiation myelopathy simulating tumor. *Arch Neurol (Chic)* 79 (1971) 371.
- MESARD L, CARMODY A, MANNARINO E and RICE D. Survival after spinal cord trauma. *Arch Neurol* 35 (1978) 78.
- PAIMER J J. Radiation myelopathy. *Brain* 95 (1972) 100.
- PHILLIPS T L and BUSCHKE F. Radiation tolerance of the thoracic spinal cord. *Amer J Roentgenol* 104 (1969) 656.
- REINHOLD H S, JOS G A, KAALEN H and UNICHT G. Radiation myelopathy of the thoracic spinal cord. *Int J Radiat Oncol Biol Phys* 1 (1976) 61.
- SEITZ D and KAIM H. Zur klinischen Differentialdiagnostik.

- nostik spinaler Rontgen Spatschaden und intramedulärer Geschwulstabsiedlungen Dtsch Z Nervenheilk 187 (1961) 155
- NER W Strahlenspatschaden des Rückenmarkes Strahlentherapie 125 (1964) 219
- OLHEIM P Radiation injury to the spinal cord Acta radiol Ther Phys Biol 10 (1971) 474
- ARA W M PHILLIPS T L SHFLINE G E and SCHWADE J G Radiation tolerance of the spinal cord Cancer 35 (1975) 1558
- ZEMAN W Histologic events during the latent interval in radiation injury In The central nervous system Monograph No 9 p 184 Edited by O T Bailey and D E Smith International Academy of Pathology 1968
- and SHIDNIA H Post therapeutic radiation injuries of the nervous system J Neurol 212 (1976) 107

CHROMOSOME COUNTS OF ^{90}Sr INDUCED OSTEOSARCOMAS IN MICE

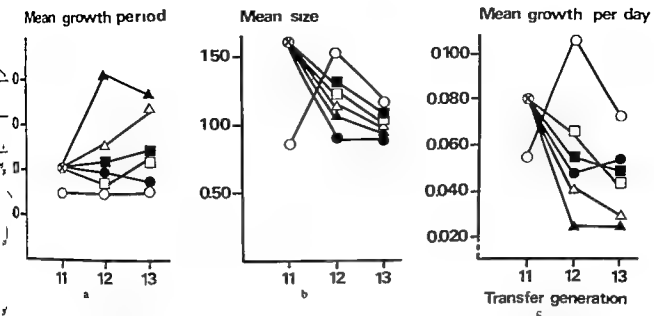
IV Variation of chromosome counts when using tumours of predetermined age for transplantation

H BERGMAN

When analysing the chromosomal progression of solid tumours a critical point may be whether a direct method can be applied or whether it becomes necessary to use a sometimes less reliable *in vitro* technique (SANDBERG *et coll* 1961 MARK 1967 SU & KELLOGG 1969). Thus in a previous report on a ^{90}Sr induced osteosarcoma quite different chromosome patterns were recorded when *in vivo* tumour transplantation was performed in parallel with *in vitro* cultures (Part III BERGMAN 1980). However it must be realized that the use of an *in vivo* method may also involve events that affect the results. As found previously in an *in vivo* parallel examination of two transplanted tumour series obtained from two tumours with different outgrowth periods of the second transfer generation of a ^{90}Sr induced osteosarcoma (Part II BERGMAN 1980) a connection between the tumours used for transplantation, the age of the tumours used for chromosome analysis and the resulting chromosome patterns could not be eliminated. The main difference consisted in a different chromosomal progression within the triploid region and a variation of the percentage of 40-chromosome cells. For this reason a primary intention of the present investigation was to examine whether a variation in chromosome count could be observed when repeated transplantations were performed from different tumours of a transfer generation. The ages of the tumours used for transplantation were predetermined.

Material and Methods

Transplantation procedures Highly inbred 60 ± 5 days old male mice of the CBA strain were used. The primary osteosarcoma with an induction time of 403 days was induced with 14.8 kBq $^{90}\text{Sr}(\text{NO}_3)_2$ /g body weight. By serial *in vivo* transplantation (NILSSON & ULLBERG 1962) the numerical chromosome progression of the original fast growing transfer series designated II had been analysed previously (Part I BERGMAN & NILSSON 1980). A tumour from transfer generation B2 was used to establish a separate slow growing tumour series b (Part II). From transfer generation b10 of this series two tumour pools constituting transfer generations b11₍₁₉₎ and B11₍₂₄₎ were established. The figures within parentheses indicate the age of the tumour used for transplantation to establish these two parallel generations. The b11₍₁₉₎ generation is not further dealt with in the present context but will constitute the origin of an investigation of tumour transplantation to hyperimmunized mice. In the present investigation repeated transplantation was carried out from transfer generation B11₍₂₄₎ (Fig. 1) on predetermined days i.e. tumour material in this case was harvested for transplantation on days 11, 18, 29, 44 and 63 after the day when the B11₍₂₄₎ generation was established. Thus from the five tumours used



Results

Recording of chromosome abnormalities As in previous investigations of ^{90}Sr induced transplanted tumours the recording of abnormalities was limited to numerical chromosome deviations and the occurrence of metacentric configurations (Parts I & II). Thus frequently appearing temporary structural abnormalities were not recorded.

A diagram of the transplantations is presented in Fig 1 and the mean outgrowth mean size and mean growth per day of the tumours per transfer generation in Fig 2. The percentage of metacentrics appears in Fig 3 while Figs 4 to 6 show cells with different chromosome numbers. The percentile chromosome distribution per transfer generation is presented in the Table.

Table (cont.)

[illegible]

Chromosome distribution: The tumours of the original B series (transfer generations B3-B10) (Part I) were primarily characterized by a varying percentage of 40 chromosome cells (22.4-73.6%) in combination with cells within the triploid region. Within this region a predominating number of 58 to 63 chromosome cells was found.

The transfer generations b3-b10 obtained by separate serial transplantations from a tumour of transfer generation B2 (Part II) differed from the tumours of the B series within the triploid region. Cells with almost continuously lower chromosome numbers were recorded. The changed predominance from 57 chromosome cells in generation b3 to 55 to 54-chromosome cells in b10. Furthermore the b generations displayed an often lower percentage of 40 chromosome cells (4.2-59%) than the B generations.

Transfer generation b11₍₂₄₎, constituting a tumour pool for the five consecutive transplantations displayed a predominant number of cells within the 51 to 54 region and with peaks at 53 (46.4%) and 54 (32%). Besides this distribution 16 per cent 40 chromosome cells were found. In contrast to these results the original B series showed a predominance of 40 chromosome cells (86.4%) and a small number of cells within the 60 to 62 region.

A certain difference is found between the five b12 generations with the varying percentage of 40-chromosome cells. The percentage of 40-chromosome cells gradually declined from transfer generation b12₍₁₁₎ (28.0%) to generation b12₍₄₄₎ (10.4% Table). The b12₍₆₃₎ generation diverged slightly from this pattern as it was characterized by 14.4 per cent 40-chromosome cells. It was also observed that while the b12₍₁₁₎ generation displayed a predominating number of cells within the 52 to 54 region the b12₍₄₄₎ generation in particular varied somewhat more i.e. from 52 to 58. The B generation was still characterized by a high frequency of 40-chromosome cells (72.8%) but also by an increased number of cells within the 60 to 62 region.

The chromosome counts of the b13 generations revealed an increased number of 40-chromosome cells. Similarly to the preceding generations (b12) most 40-chromosome cells were observed in the early transplanted generations and except for generation b13₍₄₃₎, a continuously lower percentage was observed (from 62.4% for b13₍₁₁₎ to 20.8% for b13₍₄₄₎). It was also found that the early transplanted generations still showed a narrower range of variation

Percentage of cells with metacentrics

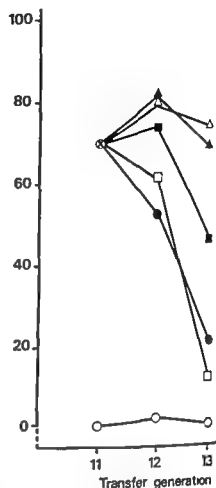


Fig. 3. Percentage of cells with one or more metacentrics in the b-series as in Fig. 2.

within the 50 to 60 region. In the B series finally continued predominance of 40-chromosome cells (67.2%) was found and within the triploid region a range of 56 to 63.

Metacentric chromosomes: The tumours of the B series displayed only 0.8 per cent cells with one or more metacentrics while within the b-series a varying percentage ranging from 11.5% (generation b13₍₁₁₎) to 80.9% (generation b12₍₄₃₎) was found (Fig. 3). Concerning transfer generations b12 from b12₍₁₁₎ to b12₍₄₄₎ the percentage increased continuously from 11.5% to 80.9% i.e. from 51.9 to 80.9 per cent. Even if a general smaller number of cells with metacentrics (11.5-74.2%) was recorded from the b13 generations the late established generations like the b17 generations showed a higher percentage. However, it must be realized that these results are biased by the number of 40-chromosome cells found. There are very rarely displayed metacentrics.

Mean outgrowth period: The tumours of the



Fig 4



Fig 5

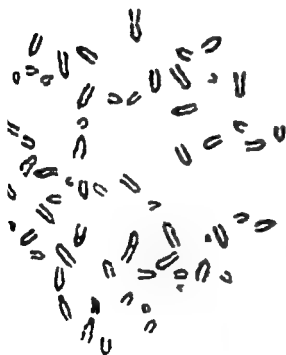


Fig 6

Fig 4 A 53 chromosome cell from a tumour of transfer generation b17.

Fig 5 A 54-chromosome cell from a tumour of transfer generation b13₄₄.

Fig 6 A 62-chromosome cell from a tumour of transfer generation B11.

several B series were characterized by the shortest mean outgrowth period per generation (14.6–16.0 days Fig 2). Regarding the b-series, a generally prolonged mean outgrowth period was observed which means that the early established generations so contained the fastest growing tumours.

Conclusions

As in previous investigations of primary tumours, fast growing tumours showed normal chromosome numbers while slower growing tumours also displayed abnormal chromosome numbers (KATO 1968, MARK 1969, MITELMAN 1971, 1972). In a previous

report on transplanted ^{90}Sr induced tumours hetero-
geneous chromosomal progression was obtained
when two tumours of a transfer generation were
used for separate serial transplantation (Part II).
An attempt has now been made to examine whether
the age of tumours used for transplantation may be
of importance for the succeeding chromosomal evo-
lution and for the outgrowth period of the trans-
plantates. For this reason repeated transplantations
were performed from different tumours of transfer
generation b11. When comparing the B and b-series
it was found that the main differences in chromosome
distribution observed in earlier transfer generations
were maintained (Part II). This means that the B
generations in general were characterized by more
40-chromosome cells and within the 50 to 62 region
a predominating number of triploid or hypertriploid
cells in contrast to the b-series which had a majori-
ty of 52 to 56-chromosome cells. Thus in spite of a
forced transplantation from early outgrown tumours
of transfer generation b11 (to obtain transfer genera-
tions b12₁₁₁ and b12₁ and then also to establish the
consecutive generations b13₁₁ and b13₁₁₀) no re-
gression could be observed to the characteristic and
stable 60 to 62 region found in the fast growing
tumours of the B series. However the early estab-
lished b12 and b13 generations displayed a higher
frequency of 40-chromosome cells and an almost
continuously lower percentage with increasing age of
the tumour used for transplantation (Table). This
result may thus indicate a connection between the
age of the tumours used for transplantation and the
chromosomal progression but it must be realized
that a quite normal accidental variation may cause
this distribution. This may also explain the variation
found within the 50 to 60 region between early and
late established generations. Even if the mean out-
growth per day and mean outgrowth period per gen-
eration (Fig. 2) should be evaluated with a certain
caution a true relationship between the age of the
tumours used for transplantation and the outgrowth
period of the transplantates cannot be eliminated.

SUMMARY

Transplanted tumours from a ^{90}Sr induced primary
tumour were used as an experimental model to eliminate
any connection between the chromosome distribution and
outgrowth period of radiation induced transplanted
tumours. For this reason repeated transplantations were

performed from different tumours of predetermined age
from a transfer generation to receive separate trans-
fer generations. From each one of these generations a
tumour of the same age as used to establish a particular
generation was used for continued transplantation. It was
then observed that the percentage of 40-chromosome
cells in general decreased with the age of the tumours used
for transplantation. Furthermore early established
transfer generations were often characterized by more
slow growing tumours. However it should be realized
that even if a true relationship between chromosome
pattern and outgrowth period cannot be eliminated the
differences found may be due to accidental variations.

ACKNOWLEDGEMENTS

The skilful technical assistance of Mrs Siva Sjöstrand and
Mr Arne Berg is gratefully acknowledged.

REFERENCES

- BERGMAN H. Chromosome counts of ^{90}Sr induced osteosarcomas in mice. II. Variation of the chromosome counts of slow and fast growing tumours in hyper and nonhyperimmunized hosts. *Acta radiol Oncology* 19 (1980) 153.
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice. III. Variation of the chromosome counts of *in vivo* transplanted tumours in *in vitro* cultures and retransplanted cultured cells. *Acta radiol Oncology* 19 (1980) 215.
- and NILSSON A. Chromosome counts of ^{90}Sr induced osteosarcomas in mice. I. Transplanted tumour series. *Acta radiol Oncology* 19 (1980) 17.
- HSU T C and KELLOGG D S. Primary cultivation and continuous propagation *in vitro* of tissues from small biopsy specimens. *J nat Cancer Inst* 25 (1969) 241.
- KATO R. The chromosomes of forty two primary Rous sarcomas of the Chinese hamster. *Hereditas* 69 (1971) 63.
- MARK J. Chromosomal analysis of Rous sarcomas in mice. Comparison between the findings in the tumour and in material explanted *in vitro*. *Acta path microbiol scand* 70 (1967) 37.
- Rous sarcomas in mice. The chromosomal progression in primary tumours. *Europ J Cancer* 9 (1969) 57.
- MITELMAN F. The chromosomes of fifty primary Rous rat sarcomas. *Hereditas* 69 (1971) 144.
- Predetermined sequential analysis of primary and metastatic Rous sarcomas in rats. *Hereditas* 70 (1972) 1.
- NILSSON A and ULLBERG S. Uptake and retention of strontium 90 in strontium 90-induced osteosarcomas. II. *Acta radiol* 58 (1962) 168.
- SANDBERG A, ISHIIHARA T, MIYATA T and HANSEN T S. The *in vivo* chromosome constitution of tumours from 34 human leukemias and 60 nonleukemic controls. *Cancer Res* 21 (1961) 678.

RADIATION SENSITIVITY OF DNA MOLECULES IN SITU
IN NORMAL AND NEOPLASTIC TISSUES OF MICE

TETSUYA ONO KIIYOHiko SAKAMOTO and SHIGEFUMI OKADA

Sensitivity to radiation in various tissues in man and in animals is known to vary a great deal depending upon the type of tissues (CLEMENTSON & NELSON 1960). In a previous report on air breathing live mice one sensitive tissue the thymus and one resistant tissue the liver were found to show similar *in situ* sensitivities in their DNA molecules (ONO & OKADA 1974). This raised the question whether all DNA molecules in all tissues of mice may have the same sensitivity regardless of the tissue sensitivity. The purpose of the present investigation was to extend the previous work into two other normal tissues (spleen cerebellum) and one malignant tissue (a squamous cell carcinoma) and to assess the role of oxygen by comparing the DNA sensitivities in various tissues from air breathing live mice and nitrogen asphyxiated dead mice. The nitrogen asphyxiated death was used to create a hypoxic state in tissues of animals as previously demonstrated by CATER (CATER & PHILLIPS 1954 CATER 1960) and used in animal experiments by DESCHNER & GRAY (1959) and HEWITT & WILSON (1959 1961).

Materials and Methods

WHT/Ht mice aged 9 to 14 weeks were used throughout the experiments. Each mouse was confined in a plastic box (40 cm×40 cm×45 cm) and irradiated at 33 Gy/min (measured by Fricke's ferrous sulfate dosimeter) with gamma rays from a 148 TBq of ¹³⁷Cs source (the Atomic Energy Research

Center University of Tokyo). For hypoxic irradiation mice were killed by nitrogen asphyxiation kept at room temperature (5–20 °C varied with the season) from 30 to 80 min (CATER & PHILLIPS CATER) to allow tissues to become hypoxic and irradiated at room temperature. Under these experimental conditions the sedimentation profiles of DNA were the same independent of the time between killing and irradiation and of room temperature.

Within 3 min after whole body irradiation tissues were removed and soaked in ice cold Fischer's medium with 10 per cent horse serum. Tissues (spleen thymus and cerebellum) were cut into pieces with a pair of scissors and sieved through stainless mesh of pore size of 125×125 microns. A cerebellar cell suspension was centrifuged once or twice at 50 G for 1 min to remove large capillary debris.

A squamous cell carcinoma transplanted subcutaneously in armpits of mice (HEWITT et al 1967 HEWITT & SAKAMOTO 1971) was used. Most tumours were from 0.1 to 0.3 g although occasionally tumours as large as 1.2 g were used and necrotic parts were removed with a pair of forceps as much as possible. The remainder of the tumour was then dispersed to prepare a cell suspension as described. Finally tumour cells were spun down at 400 \times g for 2 min and resuspended. Liver cell suspensions were prepared by the method of BRANSTER & MORTON (1957).

Per cent of total DNA

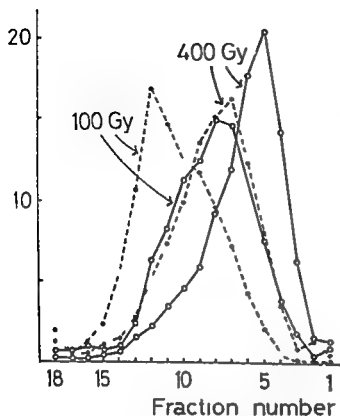


Fig. 1. Alkaline sucrose gradient sedimentation profiles of spleen DNA after 100 Gy and 400 Gy of whole body irradiation in situ under oxygenated (living) or hypoxic (dead) conditions. Sedimentation direction \Rightarrow from right to left. Solid lines with open circles indicate DNA irradiated under oxygenated conditions and the dotted lines with closed circles under hypoxic conditions. Each profile represents one spleen from one animal. The profiles here are four typical samples. The profile (not shown) of splenic DNA from a non irradiated animal overlapped with that of the profile with 100 Gy under hypoxic conditions shown in this figure.

A cell suspension was adjusted to a cell concentration of 4 to 8×10^6 cells/ml. A half ml of the suspension was gently layered over a sucrose gradient solution in a nitrocellulose centrifuge tube. The sucrose gradient was made by placing 1.5 ml of a 25 per cent sucrose solution on the bottom of a nitrocellulose centrifuge tube as a cushion followed by 34 ml of a 20 to 5 per cent sucrose gradient (0.9 M NaCl, 0.1 N NaOH and 0.01 M Na_2EDTA) and finally 0.5 ml of a lysing solution (0.5 N NaOH and 0.1 M Na_2EDTA) as a top layer (ONO & OKADA 1974). For liver cells 0.3 ml of a cell suspension was applied over 0.7 ml of the lysing solution on the sucrose gradient.

After layering a cell suspension on the sucrose gradient the gradient was kept at 29°C for 3 hours to complete lysis. Then they were centrifuged at 25000 rpm for 165 min in a SW 27 rotor in a

Beckman L3 50 Ultracentrifuge. For liver cells the centrifugation time was 270 min. The DNA sedimentation profile was analyzed by a fluorometric method (ONO & OKADA 1973) and the average molecular weight of DNA was calculated as described previously (ONO & OKADA 1974).

Results and Discussion

The alkaline sucrose gradient centrifugation patterns of splenic DNA after 100 Gy and 400 Gy of irradiation under oxic or hypoxic conditions appear in Fig. 1. The number average molecular weights of the data in Fig. 1 were 6.3×10^7 daltons under oxic and 1.1×10^7 daltons under hypoxic conditions after 100 Gy and 3.1×10^7 and 3×10^7 daltons under oxic and hypoxic conditions after 400 Gy of gamma irradiation. The sedimentation profile of the non irradiated control (not shown in Fig. 1) was very similar to that of 100 Gy under hypoxic conditions in Fig. 1 and its average molecular weight was 1.3×10^8 daltons. The reciprocals of the number average molecular weights which were proportional to the number of single strand scissions were plotted against the radiation dose (Fig. 2a). The regression analysis of normal and hypoxic experiments reveals that the efficiency of DNA breakage is 60 single strand breaks/ 10^{12} daltons DNA/Gy (or 172 eV/s b) under normal conditions and 19 single strand breaks/ 10^{12} daltons DNA/Gy (or 545 eV/s b) under hypoxia. Fig. 2b to d shows the results with thymus, cerebellum and liver. The dotted lines in Fig. 2b and 2d under oxic conditions are based on the previously reported data (ONO & OKADA 1974) with some additional points. All other experiments are new data where each point represents one tissue from one mouse.

In the liver (ONO & OKADA 1974) no data at doses over 400 Gy under normal conditions were available because most mice exposed to the higher doses died during the irradiation. The breakage efficiency of hepatic DNA under hypoxic conditions (41 s b/ 10^{12} daltons/Gy or 252 eV/s b) showed a slight reduction from those (62 s b/ 10^{12} daltons/Gy or 167 eV/s b) under normal conditions. In contrast to the results of spleen, thymus and cerebellum it is stated that the time required to rejoin 50 per cent of single strand breaks range from 40 min to 2 hours in all the normal tissues analyzed: thymus and liver (ONO & OKADA 1974) and cerebellum and liver (ONO & OKADA 1978). Thus the extent of DNA

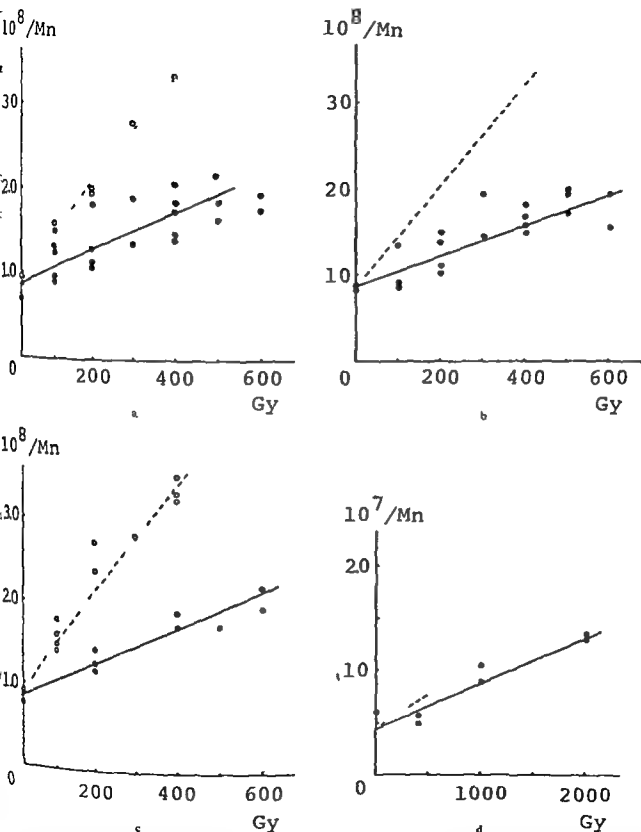


Fig. Dose response curve for single strand breaks in a) spleen b) thymus c) cerebellum and d) liver DNA in situ. The reciprocal of the number average molecular weight, which is proportional to the number of single strand breaks, is plotted against dose. Each point represents one animal. The lines represent linear regression.

curve. The dotted line with open circles is that of the oxic conditions and closed circles that of the hypoxic conditions. Dotted lines in (b) and (d) are the results of regression analysis with some additional data to the previously published curves (Oono & OKADA 1974).

Per cent of total DNA

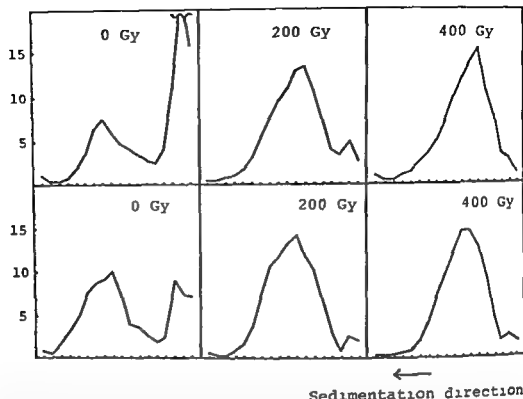


Fig 3 Sedimentation profiles of irradiated or non irradiated tumour DNA under oxic (upper curves) or hypoxic (lower curves) conditions Tumour volume was 0.1 to 0.3 g Left top panel is the sedimentation pattern of tumour DNA without a centrifugation

procedure for purification of the cell population before hypoxia cells Top three fractions were neglected for the calculation of average molecular weight

scissions rejoined during and after irradiation till death would amount at most to 8 per cent In other words the observed break efficiencies would be more than 92 per cent of the initial breaks induced by the irradiation

It is added that the DNA size in liver of non irradiated mice was found to be significantly lower than those observed in other tissues with exception of spermatozoa (ONO & OKADA 1977) Previously (ONO & OKADA 1974 1978 ONO et coll 1977) several attempts have been made to find whether autolysis of DNA during cell preparation and other steps would cause a reduction of DNA size For example DNAase inhibitor was added during the cell preparation (ONO & OKADA 1978) Or the time interval between cell preparation and cell lysis was altered (ONO & OKADA 1974 ONO et coll) None of these affected the DNA size Moreover, the DNA size of old mice was found to be smaller than that of young adult mice (ONO et coll ONO & OKADA 1978) Thus the small DNA size might be real It is also pointed out that the estimation of DNA breakage efficiency under oxic conditions is similar to those of other tissues and that the estimation of the

breakage efficiency obtained from the slope of the dose response curve would not be affected by the small DNA size of non irradiated liver

In a squamous cell carcinoma the sucrose gradient pattern of DNA was made up of one major peak in the center of the gradient and another peak near the top of the gradient The top peak represented DNA of molecular weight of less than 10^6 daltons found in the top three fractions of the gradients In this DNA content varied greatly from one sample to another A typical example is shown in the top panel of the left column of Fig 3 (oxic 0 Gy) Since the DNA may have come from autolysis of cells in the necrotic region of tumours (ONO & OKADA 1978 WILLIAMS & LITTLE 1975) a cell suspension was centrifuged at 400 G for 2 min several times before applying it to a sucrose gradient The procedure removed most of the top peaks of low molecular weight DNA (cf the rest of panels in Fig 3) When calculating the average molecular weight the top three fractions were excluded Fig 4 shows that the dose response curve of tumour DNA from live mice was slightly higher than that of nitrogen asphyxiated mice It is added that the size of tumours (0.1 g to 1.0 g)

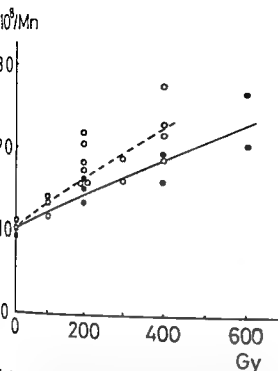


Fig. 4. Dose response curve of tumour DNA in situ under oxygenated (○) and hypoxic (●) conditions. Each point represents one normal tissue. The lines are the results of regression analysis. The standard deviations for oxic and hypoxic conditions appear in the Table.

seemed not to influence the break efficiency of tumours.

Since the rejoining time of the single strand breaks of the tumour is unavailable, the extent to which the break efficiencies estimated for tumours would be affected by a possible rejoining during and after irradiation is unknown. However, it may be speculated that the observed break efficiencies of tumours are as 70 per cent or more of the initial breaks induced by irradiation from the following considerations: (1) The rejoining rate of the tumour is as slow as those of 4 normal tissues (ONO & OKADA 1974, 1976, 1978). The observed efficiencies would represent more than 70 per cent of the initial breaks. (2) If the rejoining is as fast as that (the half time of 10 min at 100 Gy) of 4 normal tissues (the fastest among 5 tissues in this strain of mice ONO & OKADA 1976), the observed breaks would be 72 per cent of the initial breaks. (3) The carcinoma is known to contain an hypoxic component to a significant extent (HEWITT et coll. HEWITT & SAKAMOTO). Such a hypoxicity might slow the rejoining rate further (MODIG et coll. 1974). If so, more than 72 per cent of the initial breaks might be accounted for as the observed breaks.

One of the target molecules responsible for the

reproductive death of mammalian cells is probably DNA (OKADA 1971a). Several experiments have been reported in which a correlation between the DNA break efficiencies and cellular radiation sensitivity is demonstrated. Some of them are oxygen effect (MODIG et coll. LENNARTZ et coll. 1973, CHAPMAN et coll. 1974), bromodeoxyuridine sensitization of radiation (SAWADA & OKADA 1971) and protection by cysteamine and other compounds (SAWADA & OKADA 1970, ROOTS & OKADA 1972, ROOTS & SMITH 1974). Since at least two modes of cell death, reproductive and interphase death (OKADA 1971b), are known to occur in somatic cells of various tissues, this brings about the question how the sensitivity of DNA molecules in various tissues would relate to the modes of cell death. The present results are summarized in the Table. They show that DNA sensitivity in normal tissues of live mice are all in the same order of magnitude and that there is no relationship of DNA sensitivity to cellular proliferative capacity, or to tissue sensitivity or the modes of cell death.

The DNA sensitivity of tumours in mice were found to be significantly lower than those of 4 normal tissues even after a maximum correction of a possible fast rejoining.

In nitrogen asphyxiated mice, the radiation sensitivity of normal tissues with the exception of liver and of the carcinoma was found to have decreased to the same level of 17 to 22. Since nitrogen asphyxiation makes tissues hypoxic (DESCHNER & GRAY, CATER & PHILLIPS, CATER) and since the squamous cell carcinoma in situ is found to contain a significant portion of hypoxic cells (THOMLINSON & GRAY 1955, HEWITT et coll. HEWITT & SAKAMOTO), the relatively low DNA sensitivity of the squamous cell carcinoma in live mice could be attributed to its low hypoxicity. In other words, hypoxicity of the tumour seemed to be demonstrable at molecular level.

In the squamous cell carcinoma, the oxygenated portion was estimated by the TD50 method to be around 82 per cent and the hypoxic portion about 18 per cent (HEWITT et coll. HEWITT & SAKAMOTO). If it is assumed that the break efficiency of DNA in oxygenated tumour cells is 60 and 22 in hypoxic cells (Table), the observed break efficiency of 29 in carcinomas of air breathing mice would give an oxygenated component of 18 per cent and a hypoxic component of 82 per cent. The ratio of the oxygenated versus hypoxic components by the

Table

Comparison of DNA radiation sensitivity in various tissues of the mouse under oxic and hypoxic conditions. DNA radiation sensitivity in eV/s b units can be obtained by dividing 10360 with the number of s b /10¹² daltons of DNA/Gy

Organ	Number of single strand breaks/10 ¹² daltons of DNA/Gy (±SD)		OER (±SD)
	Oxic	Hypoxic	
Spleen	60±7	19±1	3.2±0.4
Thymus	59±5	17±3	3.6±0.7
Cerebellum	58±11	21±3	2.8±0.5
Liver	67±16	41±4	1.5±0.4
Tumour	29±4	27±4	1.3±0.3

TD50 method seems to be quite different from the values estimated by the present DNA break efficiency assay. One possible explanation for the difference is that the TD50 method is based upon the clonogenicity of tumour cells while the DNA sedimentation method would include clonogenic and non-clonogenic cells for their measurement. The difference in the estimation of oxygenated and hypoxic components by two methods may be evaluated by assuming a high cloning efficiency of the oxygenated component versus a low cloning efficiency of the hypoxic component. For example, if a relative cloning efficiency of 1 is assigned to the oxygenated component, the relative cloning efficiency of the hypoxic component would be 0.048 (0.18×0.18/0.82×0.82) or 4.8 per cent of the oxic component.

The oxygen enhancement ratios of normal tissues were estimated from comparison of DNA sensitivity of air breathing mice and nitrogen asphyxiated dead mice. The oxygen enhancement ratios of spleen, thymus and cerebellum were found to be about three (Table). They were comparable to the ratios estimated from DNA single strand scissions in cultured mammalian cells (CHAPMAN et coll., MODIG et coll.) rat thymocytes in vitro (LENNARTZ et coll.) and mouse testicular cells in vitro (ONO & OKADA 1976). The oxygen enhancement ratios of tissues in situ have been previously estimated only in a limited number of tissues in which either clonogenicity or cell loss could be measured. For example, intestinal crypt cells gave a ratio of 2.6 (HORNSEY 1970), the spleen 1.1 (HORNSEY 1967), the testis 1.8 to 2.5 (HORNSEY et coll. 1971) etc. The present method utilizing alkaline sucrose gradient centrifugation of

fers another means to estimate the oxygen enhancement ratios of various tissues in situ. This method can be applied to any tissue in which the present sucrose gradient method is applicable.

A low oxygen enhancement ratio of liver is somewhat different from those of tumour and testis (ONO & OKADA 1976, 1977). The low ratio in liver is attributed to a high DNA scission efficiency of the hypoxic condition, while those of tumour and testis are attributed to low efficiencies under the oxic conditions. It is possible that the liver, being full of red blood cells, would be one organ difficult to make hypoxic.

SUMMARY

The sensitivity to radiation of DNA molecules in situ in various tissues was estimated by alkaline sucrose gradient centrifugation. The sensitivity of DNA in liver, thymus, spleen and cerebellum was found to be of the same order of magnitude. The oxygen enhancement ratios of DNA in spleen, thymus and cerebellum were all approximately three. Sensitivity of DNA molecules in the tumour was about half of that in normal tissues, probably because of its hypoxicity.

ACKNOWLEDGEMENTS

The authors thank Mr T. Takatori, a medical student at the University of Tokyo, for showing that squamous cell carcinoma can be subjected to alkaline sucrose gradient centrifugation. The investigation was supported in part by the grants from the Ministry of Education in Japan.

REFERENCES

- BRANSTER M. V. and MORTON R. K. Isolation of intact liver cells. *Nature* 180 (1957) 1283.
- CATER D. H. Oxygen tension and oxidation-reduction potentials in living tissues. *Progr. in Biophys.* 10 (1960) 153.
- and PHILLIPS A. F. Measurement of electrode potentials in living and dead tissues. *Nature* 174 (1954) 111.
- CHAPMAN J. D., DUGLE D. L., REUVERS A. P., MENTEN B. E. and BORSA J. Studies on the radiosensitivity and effect of oxygen in Chinese hamster cells. *Int. J. Radiat. Biol.* 26 (1974) 387.
- CLEMONSON C. J. and NELSON A. General biology of the adult organism. In *Mechanisms in radiobiology*. Vol. 5. Multicellular organisms, p. 95. Edited by M. Frenkel and A. Forsberg. Academic Press, New York, 1970.
- DESCHNER E. E. and GRAY L. H. Influence of oxygen tension on X-ray induced chromosomal damage in Ehrlich ascites tumour cells irradiated in vitro and in vivo. *Radiat. Res.* 11 (1959) 115.

- JENNITT H B and SAKAMOTO K. The comparative survival of clonogenic cells of a murine epithelioma after irradiation in mice breathing air, oxygen and carbon dioxide or hyperbaric oxygen. *Brit J Radiol* 44 (1971) 457
- and WILSON C W. The effect of tissue oxygen tension on the radiosensitivity of leukaemia cells irradiation in situ in the livers of leukaemic mice. *Brit J Cancer* 13 (1959) 675
- Survival curves for tumour cells irradiated in vivo. *Ann N Y Acad Sci* 95 (1961) 818
- CHAN D P and BLAKE E H. Survival curves for clonogenic cells of a murine keratinizing squamous carcinoma irradiated in vivo or under hypoxic conditions. *Int J Radiat Biol* 12 (1967) 535
- KONSEY S. The recovery process in organized tissue. In *Radiation research* p 587. Edited by G. Silini. North Holland, Amsterdam 1967
- The effect of hypoxia on the sensitivity of the epithelial cells of the jejunum. *Int J Radiat Biol* 18 (1970) 539
- BRYANT P E and HEDGES M J. The effect on the sensitivity of the mouse testis of different oxygen tensions during irradiation. *Int J Radiat Biol* 19 (1971) 71
- ENHARTZ M, COQUERELLE T and HAGEN U. Effect of oxygen on DNA strand breaks in irradiated thymocytes. *Int J Radiat Biol* 24 (1973) 621
- RODIGH G, EDGREN M and RÉVESZ L. Dual effect of oxygen on the induction and repair of single strand breaks in the DNA of X irradiated mammalian cells. *Int J Radiat Biol* 26 (1974) 341
- OKADA S (a) DNA as target molecule responsible for cell killing. In *Radiation biochemistry I*. Cells p 103. Edited by K. I. Altman, G. H. Gerber and S. Okada. Academic Press, New York 1971
- (b) Radiation induced death. In *Radiation Biochemistry I*. Cells p 247. Edited by K. I. Altman, G. H. Gerber and S. Okada. Academic Press, New York 1971
- ONO T and OKADA S. An alkaline sucrose gradient centrifugation method applicable to non dividing and slowly dividing cells. *J Radiat Res* 14 (1973) 204
- Estimation in vivo of DNA strand breaks and their rejoining in thymus and liver of mouse. *Int J Radiat Biol* 25 (1974) 291
- Radiation induced DNA scissions and their rejoining in testicular cells of mouse. *Mutat Res* 36 (1976) 213
- Radiation induced DNA single strand scission and its rejoining in spermatogonia and spermatozoa of mouse. *Mutat Res* 43 (1977) 25
- Does repair capacity to rejoin radiation induced DNA breaks decline in senescent mice? *Int J Radiat Biol* 33 (1978) 403
- and SUGAHARA T. Comparative studies of DNA size in various tissues of mice during the aging process. *Exp Geront* 11 (1977) 127
- ROOTS H and OKADA S. Protection of DNA molecules of cultured mammalian cells from radiation induced single strand scission by various alcohols and SH compounds. *Int J Radiat Biol* 21 (1972) 329
- and SMITH K C. On the nature of the oxygen effect on X ray induced DNA single strand breaks in mammalian cells. *Int J Radiat Biol* 26 (1974) 467
- SAWADA S and OKADA S. Cysteamine cystamine and single strand breaks of DNA in cultured mammalian cells. *Radiat Res* 44 (1970) 116
- Effect of BUdR labelling on radiation induced DNA breakage and subsequent rejoining in culture mammalian cells. *Int J Radiat Biol* 21 (1972) 599
- THOMLINSON R H and GRAY L H. The histological structure of some human lung cancers and possible implications for radiotherapy. *Brit J Cancer* 9 (1955) 539
- WILLIAMS J R and LITTLE J B. Association of mammalian cell death with a specific endonucleolytic degradation of DNA. *Nature* 252 (1975) 754

INDUCTION OF NEOPLASIA BY ^{140}Ba IN MICE

A NILSSON P BIERKE and A BROOMÉ KARLSSON

The uptake and retention of ^{137}Ba and ^{140}Ba - ^{140}La in mouse tissues was investigated by DENCKER et al (1976). It was found that the initial distribution of radiobarium was similar to that of ^{90}Sr with a high but less specific uptake in the skeleton. This was evidenced by the fact that barium still could be autoradiographically discernible in numerous soft tissues after 2 days or more whereas strontium already after 4 hours is observable only in the hard tissues (NILSSON & ULLBERG 1962). In pigmented tissues the Ba activity seemed to be almost as high and long lasting as in the skeleton. Except for these differences radiobarium and radiostrontium have an almost identical distribution particularly within the skeleton.

Unlike ^{90}Sr ^{140}Ba has a very short physical half life (12.8 days versus 28 years) therefore offering a good opportunity for evaluating the cancerogenicity of a relatively short initial irradiation as compared with nuclides giving a life long irradiation. On the assumption that a minimum time and thus a minimum accumulated dose is necessary for induction of histologic changes successively leading to tumours it seems probable that the dose delivered after the time when autonomy has been acquired is of little importance or may even have a depressing effect on tumour development. The role of this wasted irradiation in tumour induction after contamination with long lived nuclides is debated and not fully understood. The purpose of the present communication is to give more information about the tumour incidence in relation to dose and sex after treatment

with ^{140}Ba and a better knowledge about the role of wasted irradiation for the frequency and multicentric genesis of osteosarcomas.

Material and Methods

Male and female C_{57}Bl mice (bred at the Swedish Institute of National Defence Sundbyberg) were injected intraperitoneally with ^{140}Ba nitrate when 75 ± 5 days old and weighing 25 ± 2 and 23 ± 2 g respectively. Three different doses—55.5 (1.5), 37.0 (1.0) and 18.5 (0.5) kBq (μCi)/g body weight—were used plus untreated controls as recorded in Table I. The mice were housed in conventional animal rooms 10 in each cage with free access to a pelleted diet (Standard Feed for Rats and Mice Astra Ewos Sodertälje, Sweden) and water. The room temperature was 22 to 24°C, relative humidity 45 to 55 per cent and the light/dark cycle 12/12 h. Visual health control was performed twice daily and moribund animals examined roentgenologically in dorso-ventral position. The films were used as a guide for locating bone tumours at the post mortem. The carcasses were weighed as well as the thymus, spleen, adrenal glands, liver and the testes. These organs as well as neoplastic tissues and both femora, tibiae, humeri, sternum, pelvic bones and the spine were fixed in Steeve's fluid. The bones were decalcified in formic acid 20%. Ordinary histologic techniques were used and all sections were stained according to

Table 1

C₃B₁ mice injected intraperitoneally with ¹⁴⁰Ba nitrate

Inj. amount of ¹⁴⁰ Ba/g b w		Males		Females	
		No	Group	No	Group
kBq	(μ Ci)				
55.5	(1.5)	50	A	50	II
37.0	(1.0)	50	C	50	D
18.5	(0.5)	50	E	50	F
0.0	(0.0)	70	J	70	K

the van Gieson method and with haematoxylin and eosin. All tumours were classified in agreement with the nomenclature recommended by the Committee on Pathology of the European Late Effects Project Group (EELP).

Results

The number of osteosarcomas and mean survival times are given in Table 2. In the highest and intermediate dose groups the tumour incidence was approximately 3 and 3 times higher in the female than in the male groups of mice whereas in the lowest dose groups the tumour rate was the same. As regards the relationship between dose and tumour rate dose dependency is evident particularly if the whole material is taken into consideration giving 32/21 and 10 osteosarcomas in the 55.5, 37 and 18.5 kBq groups respectively. On the other hand the dose does not seem to much influence the induction time. As regards the tumour multiplicity only the highest dose level indicates a tendency to the occurrence of multiple tumours (1/18) among the tumour bearing mice.

Of a total of 63 osteosarcomas in the whole material 48 (76.2%) were classified as osteoblastic osteosarcomas (Table 3). The predominance of this tumour type is a general feature in all dose groups. Besides the osteosarcomas found it should be pointed out that 3 cementifying fibromas were found in the alveolar bone of the male mice given 37 kBq. Also in the female control material two bone tumours, one osteosarcoma and one osteoma appeared. The femur and tibia as well as the lumbar vertebrae were the bones most frequently involved in tumour induction (Table 4). In the 55.5 and 37 kBq female groups 59 and 44 per cent of the tumours respectively were located in the long bones.

Lymphoreticular (LR) tumours of the material had a slightly higher incidence in female (51.4%) than male mice (41.4%). In the two highest ¹⁴⁰Ba groups the frequency of LR tumours was less or equal to that of the groups whereas the incidence in the low dose group was approximately the same or slightly higher than in the controls. Considering the site of LR tumours (Table 3) many of these involved mesenteric lymph nodes and histologically presented the characteristics of reticulum cell sarcoma type B (DUNN & DERINGER 1968).

In other cases the liver was the main organ involved together with the uterus and vagina, occasionally other organs as well. These tumours were obviously identical with reticulum cell sarcoma type A (DUNN & DERINGER). When the liver was the only organ affected the diagnosis of reticulum cell sarcoma was preferred. Malignant lymphomas were sometimes found which only involved the thymus.

Discussion

In a previous investigation of ¹⁴⁰Ba exposure (DENCKER *et al.*) the following approximate radiation doses were found: sternum 0.061 Gy (6.1 rad) and lumbar vertebrae 0.101 Gy (10.1 rad) per kBq of ¹⁴⁰Ba injected intraperitoneally. In these figures are inserted in order to calculate approximate irradiation doses which were calculated in femur, lumbar vertebrae and sternum for the 3 different dose groups of ¹⁴⁰Ba used in the present investigation.

As stated by DENCKER *et al.* ¹⁴⁰Ba produced bone tumours at significantly lower dose levels than observed for ⁹⁰Sr in the CBA mouse. In the present dose groups (55.5 kBq) i.e. the groups which were exposed to a dose of 6 Gy to the femur, 3.6 Gy to the lumbar vertebrae and 2.2 Gy to the sternum (Table 1) 37 per cent of the females and 18 per cent of the males developed osteosarcomas. In this dose group there was a total of 32 bone tumours if males and females were taken together, of which 13 (40.6%) were located in the femora + tibiae, i.e. bones which have accumulated a dose of approximately 6 Gy. Nine (28.1%) were found in the spine, which was calculated to have received about 3.6 Gy, and no neoplasm was detected in the sternum.

In the intermediate dose groups (4.4 and

Table 2

Incidence of malignant bone tumours and lymphoreticular tumours in relation to dose of ^{140}Ba

Group of mice	Inj. amount of ^{140}Ba /g b.w.	No. of mice	Survival days (mean \pm SE)	No. of mice with osteosarcoma	Survival days (mean \pm SE)	Total No. of osteosarcomas	Lymphoreticular tumours		Survival days (mean \pm SE)
							No.	Per cent	
(N)	55.5 (1.5)	50	603 \pm 23.9	9	772 \pm 36.1	10	15	30.0	585 \pm 21.1
(F)	55.5 (1.5)	48	590 \pm 19.4	18	614 \pm 23.2	22	15	31.3	561 \pm 31.6
(M)	37 (1.0)	49	712 \pm 21.8	5	686 \pm 100.4	5	21	42.9	707 \pm 79.5
(F)	37 (1.0)	48*	604 \pm 76.3	16*	678 \pm 41.7	16	19	39.6	572 \pm 55.9
(N)	18.5 (0.5)	49	690 \pm 22.6	5	766 \pm 51.9	5	24	49.0	675 \pm 18.6
(F)	18.5 (0.5)	49	661 \pm 24.3	5	864 \pm 54.9	5	76	53.1	640 \pm 31.1
(M)	0.0 (0.0)	70	653 \pm 21.5	—	—	—	79	41.4	641 \pm 74.3
(F)	0.0 (0.0)	70	681 \pm 19.3	1	462	1	36	51.4	648 \pm 18.0

One mouse lost during the experiment

Two mice lost during the experiment

* Including one angiosarcoma of the bone marrow

Table 3

Classification and incidence of ^{140}Ba induced tumours

Type of tumour	Injected amount of ^{140}Ba kBq (μCi)/g b.w.							
	55.5 (1.5)		37 (1.0)		18.5 (0.5)		0.0	
	Male n=50	Female n=48	Male n=49	Female n=48	Male n=49	Female n=49	Male n=70	Female n=70
Osteosarcomas								
Osteoblastic	6	19	5	11	2	4	—	1
Fibroblastic	1	3	—	1	1	—	—	—
Chondroblastic	—	—	—	—	1	1	—	—
Teleangiectatic	3	—	—	—	—	—	—	—
Mixed	—	—	—	3	1	—	—	—
Angiosarcoma of bone marrow	—	—	—	1	—	—	—	—
Cementifying fibroma	—	—	3	—	—	—	—	—
Osteoma	—	—	—	—	—	—	—	1*
Lymphoreticular tumours								
Malignant lymphoma	3	7	5	5	10	3	6	8
Reticulum cell sarcoma								
Type A	3	3	4	3	4	8	6	11
Type B	8	5	9	9	7	12	12	14
Plasmacytoma	—	—	—	—	—	—	1	1
Unclassified	1	—	3	2	3	3	4	3

Osteoma in nasal bone survival time 1030 days

to the femur (lumbar vertebrae and sternum respectively) the frequency of osteosarcomas was 33.3 per cent for females and 10.2 per cent for males. Totally 21 tumours were detected of which 7 (33.3%) were sited in femur + tibia (approx. dose 4 Gy) and 9 (42.8%) in the vertebral column (approx.

dose 2.4 Gy). In addition one tumour was found in the sternum (approx. dose 1.5 Gy).

In the lowest dose group the osteosarcoma incidence was the same 10 per cent in both sexes. In these groups 3 of 10 tumours were found in femur + tibia (approx. dose 2 Gy), 4 in the spine (approx.

Table 4

Site of malignant bone tumours

Site	Site of malignant bone tumours								Total
	Injected amount of ^{140}Ba kBq (μCi)/g b w								
	55.5 (1.5)		37 (1.0)		18.5 (0.5)		0.0		
	Male n=50	Female n=48	Male n=49	Female n=48	Male n=49	Female n=49	Male n=70	Female n=70	
Long bones									
Femur	1	2	1	1	-	2	-	-	7
Tibia	-	3	-	-	-	-	-	-	3
Humerus	-	2	-	1	-	-	-	-	3
Radius	-	-	-	-	-	-	-	-	1
Femur tibia	1	1	-	5	1	-	-	-	13
	2	13	1	7	2	2	0	0	27
Pelvic bones	1	4	-	1	1	-	-	-	7
Spine									
Cervical	-	-	1	-	-	-	-	-	1
Thoracic	2	-	-	-	-	-	-	-	2
Lumbar	1	3	1	2	1	-	-	-	6
Sacrum	1	1	1	3	1	1	-	1	11
Coccyx	1	-	-	1	-	1	-	-	4
	5	4	3	6	2	2	0	1	23
Other	1	-	1	-	-	-	-	-	2
	1	1	-	-	-	-	-	-	2
	-	1	-	1	-	-	-	-	2
Total	10	22	5	16	5	5	0	1	64

dose 1.2 Gy) and one in the sternum (approx. dose 0.7 Gy).

The experimental data indicate that in the strain of mice used the vertebrae might be somewhat more inclined to develop osteosarcomas per unit dose

Table 5

Calculated approximate radiation dose in 3 different bones of mice given ^{140}Ba at different dose levels

Bone	Calculated dose (Gy) by amount of ^{140}Ba kBq/g b w		
	55.5	37.0	18.5
Femur	6.0	4.0	2.0
Lumbar vertebra	3.6	2.4	1.2
Sternum	2.2	1.5	0.7

than the long tubular bones. In the female control material the osteosarcoma rate was 1.4 per cent. The higher incidence of osteosarcomas among females agrees well with previous findings for ^{90}Sr in CBA mice (NILSSON 1962, 1967). With reservation for strain differences between C₃Bl and CBA mice it seems to be indicated that ^{140}Ba per dose unit will produce bone tumours more effectively than ^{90}Sr .

In previous experiments (NILSSON 1973) female CBA mice developed osteosarcomas only in 6 per cent at a dose level of 1.85 kBq (0.05 μCi) ^{90}Sr g b w and in 15 per cent when the dose was doubled. The majority of the tumours were sited in the femur and the irradiation dose calculated was 33 and 44 Gy respectively. In rats however STREITSON & MOSKALEV (1961) found that osteosarcoma could be induced by ^{90}Sr in a dose of 6.3 Gy.

In the present series 2 osteosarcomas were found in the femur 2 in the lumbar spine and one in the sternum at doses of 2.12 and 0.7 Gy respectively. However this comparison should be taken with much reservation since the control group of female mice had a spontaneous frequency of osteosarcomas of 14 per cent and in addition one osteoma. This is a very high figure as compared with that of the ^{140}Ba mice. If the whole material is taken into consideration 58 mice of 293 have developed osteosarcomas after doses not exceeding 6 Gy. This may indicate that the continuous irradiation from long lived nuclides to a considerable extent may be wasted i.e. delivered after the onset of autonomy. The surplus irradiation may however play a role in the multiplicity of tumours. This should be valid for ^{90}Sr through indications that the multicentric genesis of tumours as well as the tumour latency time are functions of time and the dose accumulated (NILSSON 1970). However the present results are too vague to allow a general validity of this statement also for ^{140}Ba .

As regards the location of the tumours in various zones no definite difference related to the dose was discernible which was reported to be the case with ^{90}Sr (NILSSON 1970). The reason for this seems to be that at high doses of ^{90}Sr will continuously depress or kill the cells at sites where tumours most likely will appear when optimum cancerogenic doses are used. Initially ^{140}Ba will give a high dose to the most exposed sites but due to its short half life surplus irradiation will not interfere with reparative and proliferative events or malignant transformations of cells.

In the present series rather a decreased incidence was found in contrast to the increased rate of LR tumours reported by STRELTSOVA & MOSKALEV. This discrepancy seems largely to be explained by the very high spontaneous frequency of LR tumours in the present strain of C_{57}Bl mice.

SUMMARY

C_{57}Bl mice of both sexes were given intraperitoneal injections of ^{140}Ba nitrate at 3 different dose levels (55.5, 37.0 and 18.5 kBq/g b.w.). Osteosarcomas were found in all groups and the osteoblastic type was the most common. In the middle and the highest dose groups female mice developed tumours more frequently than the males. The irradiation doses were calculated to the femur, lumbar vertebrae and sternum and turned out to vary between 6 and 0.7 Gy.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Swedish Cancer Society for the support of this investigation (Project No. 790 B77-03X.C). The investigation was also carried out as part of the program of the European Late Effects Project Group (EULEP).

REFERENCES

- DECKER L., NILSSON A., RÖNNBACK C. and WALINDER G. Uptake and retention of ^{140}Ba and ^{140}La in mouse tissues. *Acta radiol. Ther. Phys. Biol.* 15 (1976) 273.
- DUNN T. II and DERINGER M. K. Reticulum cell neoplasm type B or the Hodgkin's like lesion of the mouse. *J. nat. Cancer Inst.* 40 (1968) 771.
- NILSSON A. ^{90}Sr induced osteosarcomas. *Acta vet. scand.* 3 (1962) 127.
- Influence of gestation and lactation on radiostrontium induced malignancies in mice. *Acta radiol. Ther. Phys. Biol.* 6 (1967) 33.
- Pathologic effects of different doses of radiostrontium in mice. Dose effect relationship in ^{90}Sr induced bone tumours. *Acta radiol. Ther. Phys. Biol.* 11 (1970) 155.
- Unpublished observations (1973).
- and ULLBERG S. Uptake and retention of strontium 90 in mouse tissues studied by whole animal autoradiography and impulse counting. *Acta radiol.* 58 (1962) 81.
- STRELTSOVA V. N. and MOSKALEV Y. I. Biological effects of barium 140 in rats. *Atomic Energy Commission translation* 7512 p. 236 (1961) (Translated from *Raspredeleme biologicheskoe deistvie i migratsiya radioaktivnykh izotopov*, Moscow 1961).

INFLUENCE OF THYROID HORMONES ON ERYTHROPOIESIS
AND RADIATION RESISTANCE IN C 57 BLACK MICE

J. MIKESKA, M. POSPIŠIL, J. NETIKOVÁ and B. HOŠEK

The relationship between the activity of hemopoietic tissue and its sensitivity to irradiation has been discussed in several reports. Animals whose erythropoiesis had been stimulated by exposure to hypoxia (TRIBUKAIT & FORSSBERG 1971) by bleeding (SMITH & WILLARD 1971) or by injection of penylhydrazine (SMITH & KINLEY 1972) showed for a certain period after irradiation a higher resistance to radiation. It is presumed that in these situations the increased erythropoiesis is associated with an increase in the number of hemopoietic stem cells, thus facilitating faster recovery from postirradiation marrow depression. Previously the possibility of stimulating haemopoiesis and the resistance to radiation of an organ by the application of thyroid hormones before irradiation was indicated in reports by POSPIŠIL et al. (1974), POSPIŠIL & NETIKOVÁ (1974) and POSPIŠIL et al. (1975). The practical possibilities of the use of hormone treatment to influence the resistance of an organism led to an extension of the experiments and further analysis of the mechanisms of these relationships. The results now published deal with the effect of thyroid hormones administered during 10 days on the resistance of C 57 Black mice. An attempt is also made to correlate the biologic effect obtained with the dynamics of influencing certain metabolic and haemopoietic functions.

Methods

Experimental animals and treatment procedure
Black mice of the inbred strain C 57 BL/6J, 10 to 12

weeks old with an average body weight of 25 to 30 g were used. They were caged in groups of 20 animals. Tap water and basal standard diet supplemented with dried pulverized thyroid gland (Thyreoidin Spofa) were given ad libitum. Thyroid gland was incorporated into the pellets by food—drug trituration at a level of 6 mg/g of food (0.6%) and administered to the mice for a period of 10 days. Body weights of the animals were recorded and group food intakes were assessed according to differences.

Irradiation The mice were irradiated with a TUR apparatus operated at 180 kV, 15 mA, filtration 0.5 mm Cu and 0.5 mm Al, dose rate 0.46 Gy/min. Single whole body doses were applied in the morning hours.

Metabolic rate The oxygen consumption was measured individually using an automatic gas analyzer operating on an open circuit principle (HOŠEK & CHLUMECKÝ 1967). During measurement the mouse was kept in a small chamber 0.5 l in volume, ventilated with atmospheric air and maintained at 23°C. The data of oxygen consumption given in the results are means of the values obtained between the 31st and 60th minute of the test and are expressed as ml/g of body weight/hour.

The amount of iodine bound to proteins of the blood serum was determined according to the method of TŮMA (1975). Blood samples were obtained by withdrawal from the axillary veins of ether anesthetized mice.

Peripheral and bone marrow cell counts Peripheral red and white blood cells and nucleated

Table 1

Erythrocyte, lymphocyte and granulocyte (before and after endotoxin) counts per mm³ of venous blood determined 14 days after sublethal irradiation with 4.8 Gy. Irradiation of the mice pretreated with thyroid was performed on various days of the rebound. 10 to 20 animals per group

	Erythrocytes ($\times 10^6$)	Lymphocytes ($\times 10^3$)	Granulocytes ($\times 10^3$)	
			Before endotoxin	After endotoxin
Control	7.39 \pm 0.17	1.59 \pm 0.09	0.90 \pm 0.07	4.00 \pm 0.39
Day of rebound				
0	7.65 \pm 0.24	1.49 \pm 0.11	0.81 \pm 0.07	3.34 \pm 0.55
1	8.23 \pm 0.19	2.06 \pm 0.23	1.06 \pm 0.11	5.17 \pm 0.48
2	8.50 \pm 0.18	1.29 \pm 0.18	1.90 \pm 0.31	11.39 \pm 0.40
3	9.15 \pm 0.18	7.84 \pm 0.74	1.76 \pm 0.78	9.66 \pm 1.02
4	8.42 \pm 0.16	2.45 \pm 0.38	1.65 \pm 0.16 *	9.68 \pm 1.64
5	8.33 \pm 0.15	2.42 \pm 0.26 *	1.50 \pm 0.17	5.99 \pm 0.95

Difference from controls: * $p < 0.05$ * $p < 0.01$

Table 2

⁵⁵Fe incorporation into heme of spleen (in percentages of injected activity), wet spleen weights and nucleated cells per femur in control mice and in animals from various intervals of thyroid treatment and rebound. 6 to 11 animals per group

	⁵⁵ Fe/spleen heme (per cent)	Spleen weight (mg)	Nucleated cells ($\times 10^3$)/femur
Control	1.04 \pm 0.17	77.9 \pm 2.6	1.38 \pm 0.04
Day of thyroid treatment			
7	1.77 \pm 0.29	108.4 \pm 6.0	1.45 \pm 0.04
10	4.56 \pm 0.62	150.9 \pm 6.7 *	1.68 \pm 0.09
Day of rebound			
1	5.24 \pm 0.66	149.6 \pm 5.3	—
2	4.79 \pm 0.29	155.1 \pm 7.9	1.62 \pm 0.08
3	5.71 \pm 0.50	141.1 \pm 8.2	—
4	2.27 \pm 0.48	96.7 \pm 5.6 *	1.84 \pm 0.04
5	2.30 \pm 0.45	98.6 \pm 5.4	1.84 \pm 0.06
11	1.38 \pm 0.20	84.5 \pm 4.0	—

Difference from controls: * $p < 0.05$ * $p < 0.01$

consumption of oxygen increased in the experimental group 2 days after the start of thyroid administration by about 30 per cent, maintaining these values during the remainder of the 10-day treatment period and falling to the level of the controls during rebound (Fig. 1). The level of protein-bound iodine in the serum during thyroid administration is an indirect indication of an increased level of thyroid hormones in the blood and has a parallel course to the oxygen consumption (Fig. 1).

Fig. 2 gives the results of experiments comparing the survival of animals irradiated with a dose of 7.3 Gy administered at various times during the rebound period after the thyroid administration. The results show a slight reduction of the resistance to radiation at the time of the cessation of the administration of thyroid hormones and a subsequent increase of the resistance, peaking on the fourth day of the rebound period. The experiments to ascertain the level of red blood cells, lymphocytes and granulocytes (counts

at rest and after endotoxin mobilization) 14 days after sublethal irradiation of animals with 4.8 Gy indicate an increase in the regeneration potential of haemopoiesis among animals irradiated during the rebound period (Table 1).

The determination of the volume of red blood cells in animals on the tenth day of treatment with thyroid gland showed a significant increase in the values ($p < 0.01$) with 0.91 ± 0.05 ml/mouse ($n=10$) as opposed to the control value of 0.64 ± 0.02 ml/mouse ($n=10$) thus demonstrating high activation of erythropoiesis by the thyroid hormones. The insignificant differences in hematocrit values ($48.1 \pm 0.7\%$ for experimental animals $48.8 \pm 1.0\%$ of control animals) indicate a parallel increase in the plasma volume of treated animals.

The dynamics of the activation of erythropoiesis in the phase of hormonal treatment and in the rebound period were analysed by the determination of ^{59}Fe incorporation into the heme fraction of the spleen. The results (Table 2) indicate a strong increase in spleen erythropoiesis during thyroid hormone treatment which is maintained during the first 3 days of the rebound period and then a sharp decline to control values on the fourth day of rebound. Spleen weight behaves parallelly.

The characterization of haemopoietic progenitors in the bone marrow of animals which had undergone thyroid gland treatment was carried out by means of repopulation assays. The erythropoiesis repopulating ability and granulocyte repopulating ability of bone marrow cells of animals treated with thyroid hormones was measured after their transplantation to normal irradiated recipients. The results appear in Fig. 3. Towards the end of the period of thyroid gland administration a slight and non significant decrease occurred in the values of both repopulation assays. During the rebound period the repopulating ability of bone marrow increased and reached statistical significance in both tests on day 4, 5 and 8. The results of repopulation assays relate to a similar quantity of injected nucleated cells (10^6). The increased number of nucleated cells in the femurs of animals treated with thyroid gland (Table 2) determined in the period of the fourth and fifth days of rebound indicates that the results of erythropoiesis and granulocyte repopulating ability relate to an absolute and not merely a relative increase in the repopulating ability of the bone marrow during this period.

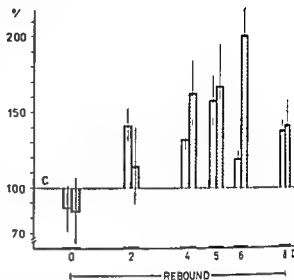


Fig. 3. Erythropoiesis (□) and granulocyte (■) repopulating ability of the bone marrow on various days of the rebound period after thyroid treatment expressed as percentages of the values of untreated controls (C). Five to 14 donors as recipients per group. Difference from controls: * $p < 0.05$; ** $p < 0.01$.

Discussion

Previously above all the radiation sensitizing effect of thyroid hormones has been demonstrated (BLOUNT & SMITH 1949; SMITH & SMITH 1951). The increased metabolic rate at the time of radiation exposure is regarded as an unfavourable factor aggravating primary radiation lesion and decreasing the radiation resistance of experimental animals. A similar mechanism may be involved in the present experiments on the irradiation of animals immediately after the discontinuation of thyroid hormone administration. An increase in resistance to radiation observed in the later period after the cessation of thyroid gland administration coincides with the adjustment of the metabolic state. In view of the fact that the consumption of oxygen does not fall during this period to below the control level the resistance cannot be considered as a protective effect of hypometabolic states (BRAUN *et al.* 1964).

Hypermetabolic conditions including hyperthyroidism lead to higher demands on oxygen supply to tissues, activate erythropoietin regulation and stimulate erythropoiesis (KRANTZ & JACOBSON 1970). The increased erythropoiesis can be associated with an increase in the total number of red cells and thus create more favourable conditions for the repopulation of the haemopoietic system after irradiation (SMITH & WILLARD FOGH). A similar mechanism may be at work in the present experiments and the effect of the thyroid pretreatment

may be similar to that obtained by the application of other factors stimulating erythropoiesis such as hypoxia (TRIBUKAIT & FORSSBERG 1966, 1969). However, in the case of the effect of hormones, the more complex stimulatory character must be taken into account. In addition, indirect calorigenic and erythropoietin activating effects of the hormones of the thyroid may also have a direct stimulatory effect on the processes of cell proliferation and differentiation (MALGOR *et coll* 1975, GOLDE *et coll* 1977).

The experiments now presented support the idea that the activation of erythropoiesis and stem cell populations is important for influencing the radiation resistance of an organism by the previous administration of thyroid gland. Erythropoiesis in the spleen, which is a particularly reactive organ of extramedullary erythropoiesis in mice (BOZZINI *et coll* 1970), demonstrates the high activity during the administration of thyroid hormones, its continuation during the period of rebound, and its rapid decline at the fourth day of rebound. The continuation of increased values of heme formation in the spleen during the first days of rebound can be explained by the accumulation of erythroid precursors due to their lower transformation output into mature cells after the reduction of demand for red cell production. Similarly, the increase of the repopulating ability of the bone marrow cells in the rebound period after thyroid pretreatment may be explained by a decreased output of cells from the undifferentiated or less differentiated stem cell compartments.

Transplantation methods evaluating erythropoietic and granulocyte repopulating ability of the bone marrow cells measure a broader part of the composition of the stem cell compartment, including both multipotent and unipotent stem cells committed to a specific differentiated pathway (GOODMAN *et coll* 1977). It is therefore not possible to determine on this basis the type of stem cell population affected by the use of the particular experimental measures. It is however possible to point out the results of FACEX *et coll* (1978) showing a rapid increase in the pool and proliferation of pluripotent stem cells (CFU) in thyroid treated mice.

The idea that an increased number of haemopoietic stem cells or a higher repopulating ability of bone marrow cells at the time of radiation risk is an important factor may not, however, be an accurate indication of the real situation. It is essential to take into account other possible mechanisms such as e.g.

changes in the radiation sensitivity of the stem cells. Unpublished results from another strain of mice did not indicate a modified radiation sensitivity of haemopoietic stem cells in thyroid pretreated animals. BERAN & TRIBUKAIT (1972) showed that the application of hypoxia to animals before irradiation does not change the degree of survival after irradiation of CFU-S. Similarly SMITH & MCKINLEY did not find any change in the sensitivity of stem cells *in vivo* after stimulation of erythropoiesis with phenylhydrazine. BERAN & TRIBUKAIT (1974) further showed that an improved growth of stem cells (CFU-S) in mice after hypoxia can also be conditioned by changes in the physiologic microenvironment. The role of similar influences in the present experimental model using hormones of the thyroid gland remains to be analysed.

SUMMARY

Oral administration of dried thyroid gland to C₅₇Bl adult male mice in a dose of 0.6 g per 100 g diet during 10 days increased the metabolic rate and stimulated the erythropoiesis. Three to six days after thyroid pretreatment the radiation resistance of the mice increased as revealed by their 30-day survival and a higher recovery of peripheral blood cell counts. The period of increased resistance to radiation was correlated with the receding of hypermetabolic effects and with the increase of repopulating abilities of the bone marrow cells.

REFERENCES

- BERAN M and TRIBUKAIT B. Haemopoietic aspects of the changes in the natural radiation resistance of mice after hypoxia. *Acta radiol Ther Phys Biol* 11 (1972) 225.
- — — Modification of the proliferative capacity of transplanted bone marrow colony forming units by changes in the host environment. *J cell Physiol* 84 (1974) 57.
- BLOUNT H C and SMITH W W. The influence of thyroid and thymic glands on mice exposed to roentgen radiation. *Science* 109 (1949) 83.
- BOZZINI C F, BARRIO RENDO M E, DEVOTO F C H and EPPER C E. Studies on medullary and extramedullary erythropoiesis in the adult mouse. *Amer J Physiol* 219 (1970) 724.
- BRÄUN H, AMMON K and HORNUNG G. Die Strahlenempfindlichkeit der Ratte bei reduziertem Grundumsatz. *Strahlentherapie* 125 (1964) 299.
- FOUGH J. Hemopoietic stem cells as a function of erythropoiesis. *Radiat Res* 45 (1971) 564.
- GOLDE D W, BERSCH N, CHOPRA I J and CLINE M J. Thyroid hormones stimulate erythropoiesis *in vitro*. *Brit J Haemat* 37 (1977) 173.

- GOODMAN R GRATE H HANNON III and HELLMAN III Hematopoietic stem cells: Effect of preirradiation bleeding and erythropoietin on thrombopoietic differentiation Blood 49 (1977) 253
- HELLMAN S GRATE H E and CHAFFEY J T Effects of radiation on the capacity of the stem cell compartment to differentiate into granulocytic and erythrocytic progeny Blood 34 (1969) 141
- HODGSON G III Erythrocyte ^{59}Fe uptake as a function of bone marrow dose injected in lethally irradiated mice Blood 19 (1962) 460
- HOŠEK B and CHLUMEČEK J Metabolic reaction and heat loss in hairless and normal mice during short term adaptation to heat and cold Pflügers Arch ges Physiol 296 (1967) 248
- KRANTZ B and JACOBSON L O Erythropoietin and the regulation of erythropoiesis The University of Chicago Press Chicago 1970
- MALGOR L A BLANC C C KLAINGER E IRIZAR S E TORALES P R and BARRIOS L Direct effects of thyroid hormones on bone marrow erythroid cells of rats Blood 45 (1975) 671
- POSPÍŠIL M and NETÍKOVÁ J Effect of pretreatment with dried thyroid gland upon white blood cell changes in sublethally x irradiated mice Fol biol (Praha) 20 (1974) 138
- — and PIPALOVÁ I The effect of dried thyroid gland pretreatment upon erythropoietic activity and development of anaemia in sublethally x irradiated mice Fol biol (Praha) 20 (1974) 9
- — HOŠEK B PIPALOVÁ I and VACEK A The effect of dried thyroid gland pretreatment upon survival formation of endogenous spleen colonies and differentiation of haemopoietic cell populations in x irradiated mice Fol biol (Praha) 21 (1975) 270
- SMITH L H and MCKINLEY T W Mechanisms of radioprotection of mice by phenylhydrazine RaEd Res 50 (1972) 611
- — and WILLARD H G Alteration of hemopoietic tissue as a factor influencing radiosensitivity of the mouse Amer J Physiol 216 (1969) 493
- SMITH W W and SMITH F Effect of thyroid hormone on radiation lethality Amer J Physiol 165 (1951) 639
- — ALDERMAN I M and CORNFELD J Granulocyte release by endotoxin in normal and irradiated mice Amer J Physiol 201 (1961) 396
- TRIBUKAIT B Über die Änderungen der natürlichen Strahlenresistenz der Maus nach mehrtägigem Aufenthalt in Hypoxie Überleben und Körpergewicht Strahlentherapie 131 (1966) 371
- — Über die Änderungen der natürlichen Strahlenresistenz der Maus nach mehrtägigem Aufenthalt in Hypoxie Funktionszustand und Strahlenreaktionen der blutbildenden Gewebe 2 Mitteilung—Erythropoiesis Strahlentherapie 138 (1969) 457
- — und FORSSBERG A Änderungen der Strahlenempfindlichkeit der Maus nach vorübergehendem Aufenthalt in Hypoxie Naturwissenschaften 51 (1964) 17
- TUMA A Photometric determination of the protein-bound iodine (In Czech) Bioch clin bohemoslov 4 (1975) 103
- VACEK A ROTKOVSKÁ D BARTONÍČKOVÁ A and POSPÍŠIL M Effect of hyperthyroidism on haemopoietic stem cell kinetics in mice Cell Tissue Kinet 11 (1978) 487
- VACHA J Blood volume in inbred strain BALB/c CBA/J and C57BL/10 mice determined by means of ^{59}Fe labeled red cells and ^{59}Fe bound to transferrin Physiol bohemoslov 24 (1974) 413
- — DUNGEL J and KLEINWACHTER V Determination of heme and non heme iron content of mouse erythropoietic organs Exp Hemat 6 (1978) 718

ELECTRON AND PHOTON BEAMS FROM A 50 MeV
RACETRACK MICROTRON

A BRAHME T KRAEPELIEN and H SVENSSON

Accelerator produced high energy electron and photon beams have been used for radiation therapy since the early fifties. The radiation modalities of most frequent use today are of low and medium energy from a few MeV up to about 20 MeV. This is the case even though for many target locations better dose distributions are obtained at considerably increased beam energies. The development and use of high energy radiation modalities with energies up to about 50 MeV have been limited by a number of factors.

One of the most important reasons is the technical problems of obtaining radiation beams of high quality at these high energies. In the case of electron beams the advantageous steep dose fall-off behind the therapeutic range is largely lost in particular if ordinary scattering foils are used to flatten the beam. For the photon beams the problem is instead that the very thick flattening filters needed will decrease the effective photon energy so that only a small improvement in beam penetration is obtained with increasing the beam energy. Limitations of almost equal significance have arisen in the absence of a small compact and light weight accelerator of sufficient output and low cost. The accelerators for example have been restrained by their low weight, low dose rate and often poor dose distributions at least for the electron beams. Similar problems with the high energy linear accelerators have been caused mainly by their large dimensions or high power requirements and costs but also by the beam

flattening problems mentioned. However the racetrack microtron which is the most recently developed electron accelerator in this energy range (VERNHOLM et al 1974) is not restricted in these respects due to its small size (1.5 m × 0.5 m × 0.4 m), light weight (1500 kg) and high beam current (20 μA at 50 MeV).

Some new methods to improve the usefulness of high energy photon and electron beams based on such an accelerator are therefore discussed. This is done not only in terms of the spatial distribution of absorbed dose but also in terms of the relative biologic effectiveness considering different fractionation patterns and grid techniques. The proposed new treatment techniques are illustrated by dose distributions measured in the research beam from a 50 MeV racetrack microtron.

Accelerator

The working principle of the racetrack microtron (Fig. 1) was suggested by SCHWINGER (quoted by SCHIFF 1946) only a few years after the principle of the conventional microtron was presented by VEKSLER (1944). The fundamental improvements gained by splitting the magnet of a conventional microtron in two equal halves are threefold.

(1) A new degree of freedom is introduced allowing the use of much higher magnetic fields resulting

in a very compact accelerator and continuously variable electron energy

(2) The field free region between the two magnet halves gives more space for the acceleration section allowing larger energy gain per revolution than is possible with a single resonator cavity

(3) The focusing of the accelerated electrons can likewise be improved in the gap between the magnets by quadrupoles or special design of the semi-circular magnets (FROELICH & BRANNEN 1967; BIRN & SIDIACEK 1967)

The development and properties of the racetrack microtron were discussed in detail by WILK & WILK (1970) and the particular machine used in the present investigation was described in more detail by WERNHOLM *et coll*. The use of an 18 or 25 MeV racetrack microtron for radiation therapy was discussed previously by MACDONALD & FROELICH (1974)

Fundamental considerations

Electron therapy The design of broad uniform electron beams of high energies and large therapeutic ranges has been discussed extensively in the literature. The basic problems involved can be subdivided into two parts. The first concerns the problems of obtaining broad flattened electron beams with uniform intensity distribution and the second concerns how the therapeutic ranges are obtained.

The most common method of beam flattening is by using a single scattering foil. This is not satisfactory except possibly for small field sizes and energies below about 10 MeV since the thick scattering foils otherwise needed degrade the electron beam quality considerably (BRAHME & SVENSSON 1976a, 1979). The dual scattering foil technique is about one order of magnitude better with regard to the amount of material in the beam and will thus give high quality electron beams at energies up to about 20 MeV (BRAHME 1977). However above this energy the beam degradation again becomes considerable. At the highest energies from about 25 MeV to 50 MeV the electromagnetic systems using defocusing or scanning magnets or quadrupoles (HSIEN & UHLMANN 1956; AUCOUTURIER *et coll* 1970) are the only possibilities for obtaining high quality beams.

Among the methods used to obtain an increased therapeutic range are linearly moving deflection magnets (ROZENFELD *et coll* 1969). These change the normal quadratic dose decrease with distance

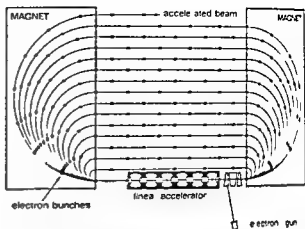


Fig. 1. Cross section through the orbital plane of the racetrack microtron used. Electrons are accelerated up to 15 times by the short linear accelerator section with a 3.3 MeV energy gain per revolution giving a maximum energy of about 50 MeV. Electrons are injected by a small deflection magnet in front of the linear accelerator.

which is obtained with a small isotropic radiation source to a linear dose decrease obtained with a line source. This will increase the therapeutic depth of the beam as the dose fall-off of the depth dose curve will be decreased.

A method which uses a similar principle is the small angle pendular technique practised with the fan beams of some betatrons (RASSOW 1970). However similar results can be obtained with most treatment units simply by applying a multitude of parallel or convergent beams. In the limit of a continuum of displaced fields (arc or moving beam therapy) the geometric dose decrease can even be transformed to an increase.

From this point of view it should be sufficient for most practical purposes to use a scanning system where the effective radiation source is small. In Fig. 2 such a system is illustrated which also is capable of scanning the photon beam. It consists of two scanning dipole magnets, each deflecting the beam in one of the orthogonal planes, and with a bending magnet in between. By using the beam optical properties of the bending magnet the first scanning dipole can be placed in front of the bending magnet with the image of its scanning centre near the middle of the second dipole, which is scanning in the opposite plane. In this way a very compact deflection system is obtained with the position of the effective radiation source coinciding for the two orthogonal planes.

A number of advantages are inherent in such a beam scanning system, not least from a beam geometric point of view. It implies a well defined

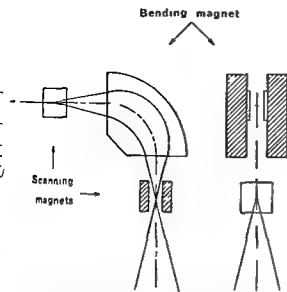


Fig. 1. Design of proposed scanning system for the electron and photon beams from an electron accelerator (two orthogonal sections). The photon beam is scanned by placing the target immediately below the last scanning magnet.

source to surface distance (SSD) and the fact that the emergent beam for most practical purposes can be described as a point isotropic beam with the same fluence and energy distribution in all directions. In addition the distortion of the elementary beam by the simple scanning dipoles will be considerably less than if a more complex quadrupole is used to scan the beam in two planes.

Photon therapy. The scanning system shown in Fig. 1 is of special interest since the second dipole can be made very strong and short so that a bremsstrahlung target can be placed close to the effective electron source and thus convert the scanned electron beam to a scanned photon beam. This is of considerable importance not only as it will make broad uniform photon beams available at any photon energy without the need of thick flattening filters but also because the photon spectrum will be improved when the degrading flattening filter is removed and the hard forward peaked photon spectrum is distributed uniformly over the entire radiation field.

The conventional methods of obtaining broad uniform photon beams have been discussed extensively in the literature. Two principle designs have been suggested either using a low atomic number target and flattening filter to produce a beam of the highest possible mean photon energy and penetration on the central axis of the beam (PODGORSKI et coll 1974) or using a thin high atomic number target with an

electron stop and a composite flattening filter to produce a photon beam of high uniformity at all depths and field sizes due to simultaneous uniformity in photon fluence and mean photon energy (BRAHME & SVENSSON 1976b). The first method will produce very penetrative narrow beams at the cost of a variation in uniformity with depth for broad beams. The second method will instead produce uniform beams practically independent of field size and depth but at the cost of a slightly reduced penetration at small field sizes.

The suggested scanned photon beam combines the advantages of the photon beam designs mentioned but avoids their respective disadvantages. Furthermore the scanned photon beam will also increase the available dose rate since a flattening filter which often absorbs as much as 50 to 80 per cent of the photon fluence is no longer needed. As a consequence the radiation shielding problems outside the field will be decreased by a factor 2 to 5 and so will the neutron production in the flattening filter and collimators. This last problem which causes much debate today particularly in the USA (cf NBS 1979) can thus be reduced considerably by using a scanned photon beam.

Radiation biology. The production of broad uniform radiation beams by scanning a narrow elementary beam has a number of consequences of biologic nature which may become of clinical value.

The radiation beam from most accelerators consists of short intense pulses of a few microseconds duration at repetition rates of about a hundred pulses per second. Therefore when the elementary beam is of small cross section each intense pulse may be placed in an orthogonal or hexagonal pattern to obtain dose distributions similar to those used in grid or sieve therapy.

GRIEM et coll (1969) reported absence of skin reaction using a pulsed scanning electron beam for radiation therapy. Their scanning system gave an almost uniform dose distribution in the superficial skin layer therefore a sieve effect was unlikely to be the explanation for the absence of reaction. They also showed in survival curves for Chinese hamster cells irradiated with two different beam currents during the pulses but with the same total absorbed doses to the cells that the high dose rate gave the smallest biologic effect. A possible explanation for this effect could be a depletion of intracellular oxygen. However a very large dose per pulse is necessary to give this effect (EPP et coll 1968).

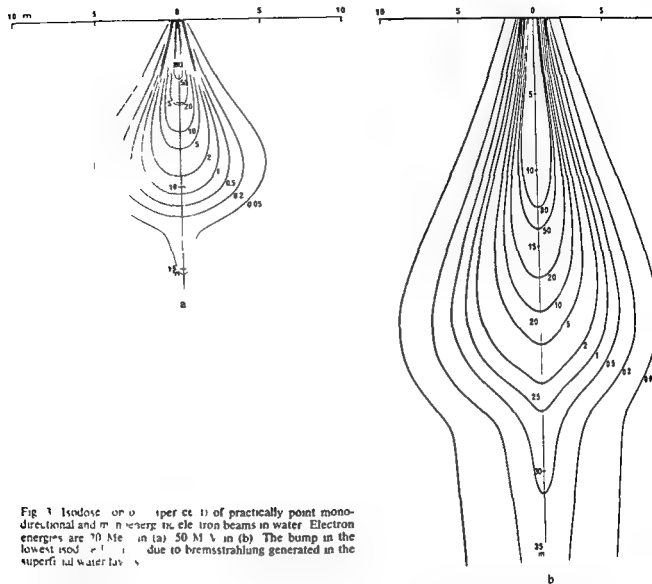


Fig 1 Isodose curves (per cent) of practically point mono-directional and mono-energy electron beams in water. Electron energies are 70 MeV in (a) 50 MeV in (b). The bump in the lowest isodose level is due to bremsstrahlung generated in the superficial water layer.

making this explanation unlikely. No conclusive explanation has yet been given.

An effect working in the opposite direction should be expected at short pulses of very high dose rate as a result of instantaneously overlapping electron tracks. This should result in an increased RBE at extremely high dose rates due to increase in effective LET. The pulse length needed for such effects to be important is about 50 ns and the dose rate in the pulse should be above 10^8 Gy/s which is a regime marginally accessible with a scanning beam system.

The sieve therapy was much analysed and used in the days of conventional roentgen therapy in order to decrease skin reactions (e.g. JOLLES (1953) BAUM (1958)) but is also stated to be justified for high energy photon beams (BECKER *et al.* 1958 ALIEV 1976). With electron radiation below about 20 MeV there seems to be no reason for grids, as the

low energy electrons are used to obtain a uniform beam in a target volume at a small depth. However, at higher electron energies the target volume is often situated at a large depth and it may be desirable to save the normal tissue at small depths. The grid technique could in such cases be very convenient as a grid effect can be obtained at small depths but a complete uniform irradiation at larger depths is not possible. With a grid technique fairly massive doses may be given to normal tissue. ALIEV used on some patients (in his series of 251 patients) more than 220 Gy without any severe reaction. No clinical or experimental investigation for high energy electrons above 20 MeV is available.

Combination effects may be gained with a scanning system. Thus the low skin reactions at high pulse and dose rate reported by GRILLI may be combined with a favourable grid technique. The reduction in the response of normal

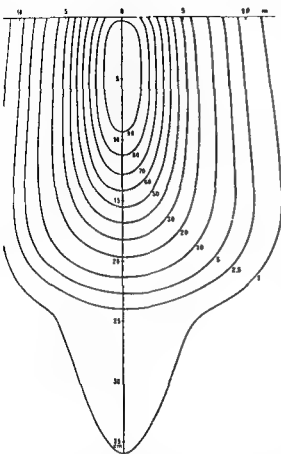


Fig. 4. Isodose contours (per cent) of a 45.6 MeV Gaussian electron beam produced by a narrow pencil beam transported through vacuum window, dose monitor and 1 m of air. Resultant beam square radius is 45.5 mm.

compared with conventional therapy at a low dose rate with uniform irradiation of the superficial tissue is impossible to estimate from presently available literature. Many of the experimental results reported in the following have been obtained in investigations of the technical, physical and biologic attributes of a scanning beam system for radiation treatments.

Electron absorbed dose distributions

Pencil beams. Due to their importance for the scanned and grid beam techniques, dose distributions from almost point monodirectional and monoenergetic electron beams have been measured. The isodose curves for such pencil beams of 20 and 40 MeV electrons are presented in Fig. 3. The dose distributions were measured in a 40 cm × 40 cm × 40 cm water phantom with a 0.1 mm mylar window and immediately after the electron beam left the scanning system through a 0.08 mm aluminum foil.

The root mean square diameter of the electron beam was less than 5 mm with practically no electrons outside a diameter of 10 mm. The angular divergence of the beam due to the emittance of the accelerator, the focusing of a weak quadrupole lens and additional scattering in the vacuum window was only a few degrees. It was essential that the electron beam was not collimated by a narrow material aperture as that would generate excessive bremsstrahlung contamination which would disturb the measurements. The energy spread of the beam was less than 0.2 MeV at the full width at half the maximum electron fluence.

The absorbed dose distribution was determined by a small silicon diode of 2 mm × 2 mm cross sectional area. The dose distributions were checked with ferrous sulphate measurements in a 40 MeV electron beam. The agreement was better than 1 per cent over the entire range of measurement from a depth of 2 to 22 cm, in good agreement with previous diode measurements at lower energies.

The absorbed dose in a pencil beam decreases very rapidly with depth as the beam is spread out in the water (Fig. 3). The isodose curves have therefore somewhat arbitrarily been normalized to 100 per cent at a depth of about 2 and 12 cm respectively.

The characteristic pear shape of the isodose curves is fundamentally due to the accumulative multiple scattering interactions of the electrons in consecutive water layers in combination with their decreasing energy. It is of interest to see how the pear shape at the lowest isodose levels is influenced by the strongly forward peaked bremsstrahlung generated in the first part of the electron tracks. It is also observed that the photon tail is more marked at the higher energies as should be expected.

Of particular interest is that the width of the dose distribution does not vary much with the electron energy. This effect is caused by the high scattering power and low penetration depth at low energies and the increasing penetration but simultaneously decreasing scattering power with increasing energies. More exactly, the range is directly proportional to the energy and the scattering power inversely proportional to the energy squared. Since the lateral deflection is approximately proportional to the product of range and root mean square deflection angle, it will thus only have a very weak energy dependence.

This general result indicates that in practice the same grid spacing could be used for sieve therapy

independent of electron energy at least when a uniform absorbed dose is desired at the treatment depth but not at the surface.

Gaussian beams A point monodirectional or pencil beam can only be used for radiation therapy if the vacuum window is thin and placed close to the patient. Even if such a system is in clinical use (ROZENFELD et al.) it is not a very practical one for routine radiation therapy. The beam normally has to be transported some distance through air after having penetrated the vacuum window and has also to pass a transmission monitor and mirror for light field indication. All these components will spread the electrons so that the electron beam reaching the patient will necessarily have a certain width. Fig. 4 shows the lateral isodose distribution of such a 46 MeV electron beam which has passed through the vacuum window (0.08 mm aluminium), 1 m of air and 3 monitor foils (0.03 mm molybdenum). This electron beam can for most practical purposes be described by a Gaussian radial distribution. From a comparison with Fig. 3 it is seen that the characteristic pear shape of the pencil beam is almost lost largely due to the influence of the bell shaped lateral dose decrease.

From these results it can be concluded that in order to obtain a long gird effect (large ratio between penumbra and background dose) the amount of material the beam should be minimized.

Broad beam Three basically different techniques have been used in the present experiments to obtain the central axis depth dose distributions for broad electron beams.

The first method used was a thin scattering foil combined with a very long source to surface distance in order to produce broad almost parallel and monoenergetic electron beams. The results appear in Fig. 5 for intervals of 10 MeV from 10 to 50 MeV at an SSD of 200 cm and a scattering foil of 0.1 mm lead. When the appropriate corrections are made for the collisional energy losses in the vacuum window, scattering foil and air, the most probable accelerator energy determined from the practical range of these curves agree within 0.3 MeV with the accelerator energy obtained from the magnetic field of the accelerator. The energy range relation used was

$$(E_p)_0 = C_1 + C_2 R_p + C_3 R_p^2 \quad (1)$$

or

$$R_p = C_1 + C_3 (E_p)_0 + C_4 (E_p)_0^2$$

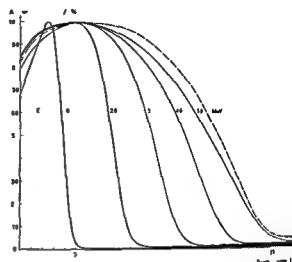


Fig. 5 Electron central axis depth dose curves produced by a scattering foil of 0.1 mm lead at a SSD of 200 cm (—). Dashed curve obtained by dual foil technique with a primary foil of 0.1 mm gold and a shaped secondary foil of 1.8 mm aluminium.

where

$$C_1 = 0.22 \text{ MeV}$$

$$C_2 = 1.98 \text{ MeV cm}^{-1}$$

$$C_3 = 0.0025 \text{ MeV cm}^{-2}$$

$$C_4 = -0.11 \text{ cm}$$

$$C_5 = 0.505 \text{ cm MeV}^{-1}$$

$$C_6 = -0.0003 \text{ cm MeV}^{-2}$$

The discrepancies observed were all within the uncertainty of the magnetic field determination. The traditional energy range formula derived by M. KUS for the energy range below 20 MeV (ICR 1972, NACP 1972) is in error by as much as 2.5 MeV at 50 MeV. Relation (1) is now recommended by the NACP (1980) for the energy range 1 to 40 MeV.

The small increase in therapeutic range above some 30 MeV even at this fairly large source to surface distance is evident. However the beam is far from broad at the highest energies. Instead it is practically Gaussian with root mean square diameters of only 16.8 cm at 50 MeV and 20.6 cm at 40 MeV.

The second method used was the dual scattering foil technique with which perfectly uniform electron beams can be produced with a minimum of scattering material (BRAHME 1977). The dashed curve in Fig. 5 was produced in this way with a primary scattering foil of 0.1 mm gold and a secondary Gaussian shaped foil of aluminium 1.8 mm thick at the centre. With this foil combination the beam is perfectly flat out to a diameter of 20 cm. A substantial improvement is seen compared with the single scattering foil beam even though the foil thickness was increased considerably. In Fig. 6 the isodose distribution

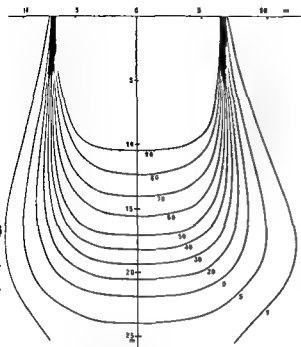


Fig 6 Isodose contours (per cent) corresponding to the dashed depth dose curve in Fig 5. Field size is 14 cm x 14 cm and accelerator energy 50 MeV

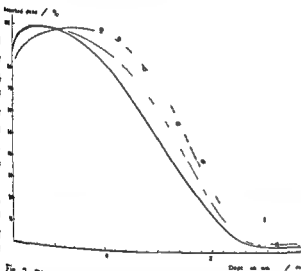


Fig 7 Electron central axis depth dose curves in water at $E_0 = 6$ MeV. Solid line corresponds to Gaussian beam in Fig 4 (SSD 100 cm, $\phi_{rms} = 9.1$ cm). Dashed curve is obtained by rectilinearly scanning the Gaussian beam to produce a plane parallel beam (SSD ∞ , $\phi_{rms} = \infty$). In the dotted curve scanning was point isotropic as would be obtained according to Fig 2 (SSD 100 cm, $\phi_{rms} = \infty$). Circles correspond to the dashed curve but with a pencil beam similar to Fig 3

tribution of this beam is shown for a 14 cm square field. It appears that uniform electron beams of fairly high quality can be obtained with the dual foil technique at intermediate field sizes and in large SSD

The third method used to obtain broad beam dose distributions is based on the integration of narrow beam isodose distributions. With this method broad beam data can be obtained by summation of a large number of narrow beams (HARDER 1965). This method could also be described as a mathematical way to simulate the broad beams produced by the rectilinear scanning of a narrow beam.

Two different sets of narrow beam data were used for this purpose: the pencil beam similar to that in Fig 3 and the Gaussian beam in Fig 4. The integration of the pencil beam isodoses was performed by fitting the lateral fluence at each depth to a curve of the type

$$D(r) = \frac{D(0)}{1 + ar^2} \quad (2)$$

The central axis absorbed dose distribution in a broad circular beam of a given radius is due to the reciprocity obtained by integrating eq (2) out to the desired beam radius.

The result for the pencil beam is shown as circles in Fig 7 for a beam diameter of 15 cm. The shape of the depth dose in the surface region could not be determined accurately in this way for the pencil beam, since this region was distorted considerably by the finite size of the detector being almost equal to the diameter of the beam. For this reason the Gaussian beam data were also used to calculate broad beam dose distributions. Due to the quite different lateral dose distribution, the lateral profiles were instead approximated by a Gaussian radial distribution.

$$D(r) = D(0) \exp(-r^2/\sigma^2) \quad (3)$$

This gave more accurate broad beam depth dose data since the finite detector size had no influence on the much wider crude beam isodose distribution of Fig 4. The resultant depth dose curve also appears in Fig 7. A fair agreement in curve shape is found between the integrated Gaussian beam (the dashed curve) and the pencil beam but the lesser accuracy of the latter is evident, particularly at small depths where the pencil beam is very narrow. A few interesting conclusions can nevertheless be drawn from its more accurate dose fall-off section. First, the range of the pencil beam is slightly longer due to the one meter of air and the monitor foils which were present when the elementary pencil beam distribution was measured. Secondly, the dose gradient is

not so good for the pencil beam curve. One possible explanation for this is that the angular spread in the pencil beam of a few degrees is sufficient to reduce the dose gradient (BRAHME & SVENSSON 1978). In the crude beam case the almost plane parallel central part of the beam is mainly used (cf Fig 4).

From the comparison of central axis depth dose distributions in Fig 7 the considerable improvement in depth dose characteristics produced by good uniformity and a long source to surface distance is evident. In order to separate these two effects the inverse square law has been included in the integral

(the dotted curve). The difference between the solid and the dotted curves on the other hand are due to the inverse square attenuation of the electron fluence which evidently is most responsible for the loss of therapeutic range at these high energies.

However it should be emphasized that both the calculated curves in Fig 7 are somewhat degraded since the Gaussian beam was not strictly parallel. Therefore the calculated plane parallel beam contains a small angular spread of a few degrees which probably has decreased its therapeutic range and dose gradient by a small amount (BRAHME & SVENSSON 1978). The point isotropic beam is somewhat more uncertain due to the approximate nature of the inverse square correction and to the fact that this was made on a beam which already contained a slight divergence.

Photon absorbed dose distributions

Pencil beams. Narrow beams of high energy photons are of interest not only for the understanding of the basic interaction processes of photon beams with matter but also for direct application in radiation therapy and surgery of deep seated organs (SARBY 1974). For these purposes it is of great value to know the depth dose and isodose curves in centimeter sized photon beams of very high energy.

The isodose curves of a 10 mm diameter photon beam of 46 MeV peak energy and a SSD of 100 cm appear in Fig 8. This beam is obtained from the central part of the unfiltered bremsstrahlung beam produced by an intermediate thickness target of 5 mm tantalum backed up by 25 mm of aluminum for electron absorption. It is immediately observed that this high photon energy is of great interest for medical use due to its narrow penumbra and large half value depth of about 19 cm.

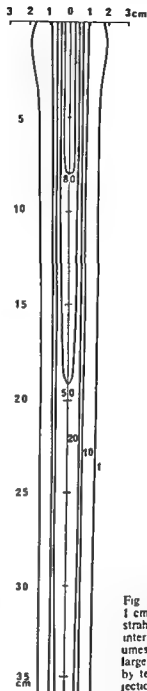


Fig 8 Isodose contours (per cent of a 1 cm diameter 46 MeV non filtered bremsstrahlung beam). This type of beam is of interest for small localized target volumes but also for dynamic treatment of larger volumes of more complicated shapes by techniques analogous to the back projection used in a CT scanner.

A number of physical properties of the energy deposition of high energy photon beams can be observed. Even though the range of the secondary electrons and positrons produced by this 46 MeV beam is quite large (almost 10 cm) they deposit nearly all their energy inside a beam with a narrow penumbra region of about 5 mm between the 80 and 20 per cent isodoses. The reason for this is that most of the secondary photons and electrons are strongly forward peaked and thus the beam acts like a continuous line source of high energy electrons (cf Fig 3) which deposit most of their energy close to the

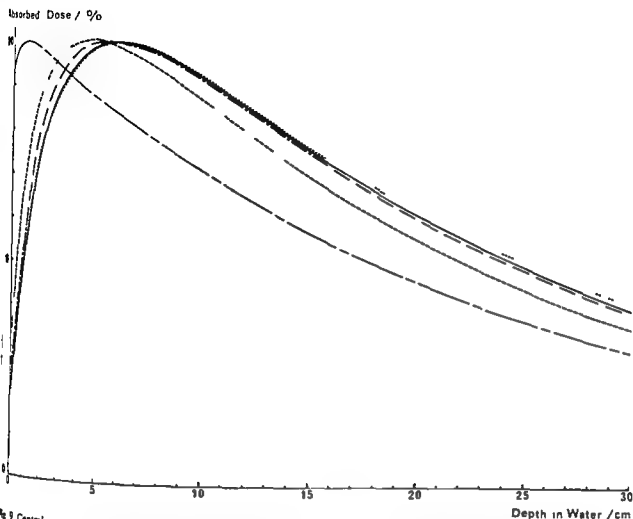


Fig. 9 Central axis depth dose curves for elementary non filtered photon beams (46 MV photons). Dashed curve with superficial dose maximum is the pencil beam of Fig. 8. Lateral dose distribu-

tion of the other curves appears in Fig. 10. Targets: 1.05 mm Ta + 21.5 cm C (---), 1.5 mm Ta + 11 cm Al (—), 9 mm Ta (---), 5 mm Ta + 2.5 cm Al (Ø 1 cm) (---). The very small dose build up seen on the central axis depth dose curve in Fig. 9 is caused by the quickly established partial equilibrium of photon generated electrons. Already after a few cm depth a balance is obtained between those electrons which are lost from the beam by multiple scattering in interactions and those generated in the beam by photon interactions. The shape of the depth dose curve is, except for the build up region, similar to the depth dose obtained in a broad beam of 10 MV photons.

Elementary photon beams. The elementary unfiltered photon beam obtained when a narrow electron beam strikes a target is of fundamental importance for the present experiments since this is the beam which will be scanned to produce broad uniform photon beams. The distribution of absorbed dose in elementary photon beams is dependent on the properties of the target. At the high energies which pres-

ently are of main interest there is only a small variation in dose rate between different target materials (PODGORSAK et coll.). However, with decreasing atomic number and target thickness the lateral width of the elementary beam will decrease considerably and the high energy photons will more and more be peaked in the forward direction.

This is evident in Fig. 10 where the lateral dose distributions at dose maximum of the elementary photon beam from a number of different targets are shown. In order to simplify extrapolations to different beam energies the lateral variable was chosen as the product of peak photon energy and angular direction from the incident electron beam. By using this variable the distributions will have a very small energy dependence as both the photon production angle ($m c / E$ of the circle in Fig. 10) and the root mean square electron scattering angle of the electrons in the target ($(\bar{\theta}^2)^{1/2}$) are inversely proportion-

ally are of main interest there is only a small variation in dose rate between different target materials (PODGORSAK et coll.). However, with decreasing atomic number and target thickness the lateral width of the elementary beam will decrease considerably and the high energy photons will more and more be peaked in the forward direction. This is evident in Fig. 10 where the lateral dose distributions at dose maximum of the elementary photon beam from a number of different targets are shown. In order to simplify extrapolations to different beam energies the lateral variable was chosen as the product of peak photon energy and angular direction from the incident electron beam. By using this variable the distributions will have a very small energy dependence as both the photon production angle ($m c / E$ of the circle in Fig. 10) and the root mean square electron scattering angle of the electrons in the target ($(\bar{\theta}^2)^{1/2}$) are inversely proportion-

Absorbed Dose / %

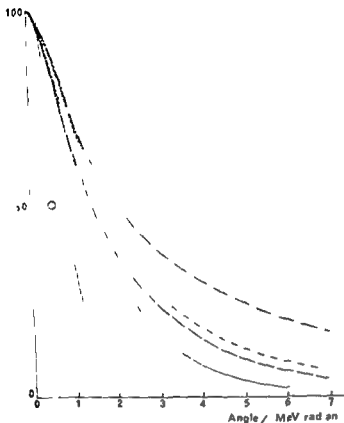


Fig 10

Fig 10 Angular dose distribution of non filtered bremsstrahlung photons from different targets. The circle indicates the opening angle of the pure bremsstrahlung process (m_0c^2/E). Targets: 9 mm Ta (—) 1.5 mm Ta+11 cm Al (---) 1.05 mm Ta+21.5 cm C (—) 21.5 cm C (---) 5 cm C (—) 9 mm Be (—) 1 mm Be (---)

Fig 11 Iso-dose contours curves (per cent) of 46 MeV elementary photon beams in water at a target to surface distance of 100 cm. For curve a) the target was 21.5 cm of graphite and for curve b) 9 mm of tantalum.

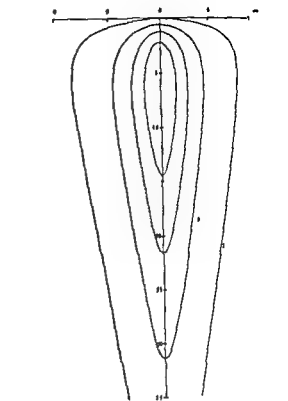


Fig 11a

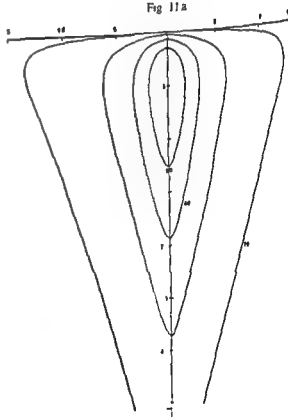


Fig 11b

nal to the energy of the incident electrons (BRAHME & SVENSSON 1979).

It should be observed that the broadest angular distributions are obtained for thick, high atomic number targets where the electron scattering con-

tribution is largest. Similarly the narrowest beams are obtained in thin targets of low atomic number where minimum influence of electron multiple scattering is obtained and the angular spread approaches that of the pure bremsstrahlung process (cf. the con-

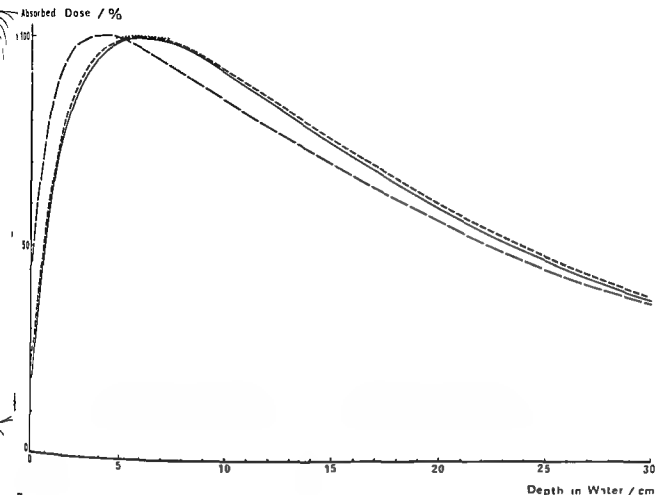


Fig 17 Central axis depth dose curves of 50 MV photons at an SSD of 100 cm. Solid line represents non filtered elementary beam. Dashed curves are filtered by titanium (4 cm ---) to a

cross section of 8 cm and by lead (4 cm —) to 30 cm respectively. Dotted curve obtained by scanning the elementary beam over a circular area of 30 cm cross section.

in Fig 10). The thin target distributions were obtained with a deflection magnet behind the target to deflect the transmitted electrons out of the useful beam. In addition the measurements were made at a large depth in water (30 cm) so that the influence of scattered electrons in the incident beam was minimized.

In Fig 9 several examples are shown of the central axis depth dose distribution in elementary photon beams. The deepest dose maximum and highest depth dose is obtained for the thin high atomic number target with the carbon electron stop and not for the pure carbon target. This somewhat surprising result is due to the narrower width of the pure carbon target beam (cf Figs 10 and 11) which nevertheless has the lowest surface dose.

The thick high atomic number target produces with large margin the least penetrative beam even though this beam is more similar to a broad beam. The reasons for this are threefold. First the radi-

ative stopping power is very high even at low electron energies therefore a considerable amount of low energy bremsstrahlung is generated by the slowing down of the electrons. Secondly the very high energy photons from the superficial layers are heavily filtered in the deeper layers in high atomic number targets whereas the low energy photons are filtered more in low atomic number targets. Thirdly the high energy photon components are spread over wider angles due to a greater influence of multiple electron scattering in a high atomic number target which again decreases the mean photon energy in the forward direction.

The isodose distributions of the elementary photon beam from a low and a high atomic number target are shown in Fig 11 at a SSD of 100 cm. This is perhaps an even more impressive demonstration of the difference in lateral spread and penetration of the elementary photon beam from different types of targets. The low atomic number target was 21.5 cm

of graphite and the high atomic number target was 9 mm of tantalum

Broad photon beams The choice of an elementary non filtered photon beam for generation of broad therapeutic photon beams will greatly depend on the method of beam flattening used. If the conventional technique with thick flattening filters is used the best choice is to use a thin high atomic number target followed by a low atomic number electron stopper (BRAHME 1975) in order to spread out the high photon energy component as much as possible (cf Fig 10). The effect of this method is illustrated in Fig 12 in which a 1.5 mm tantalum target is followed by a 70 mm aluminium electron stopper. High atomic number flattening filters are necessary to obtain broad beams at high energies since the thickness of the filter otherwise would be unacceptable. The effect of a 40 mm lead filter used to produce a flattened beam of about 30 cm in diameter is illustrated in Fig 12. It is immediately seen that the beam penetration is decreased considerably by this filter even compared with the elementary beam. The reason for this is the considerable loss of high energy photons and consequent contamination of the beam with lower energy annihilation quanta and scattered photons. For smaller field sizes intermediate thickness filters may also be used. A 4 cm thick filter was consequently used to produce the elementary beam as also appears in Fig 11. The dose is now improved somewhat compared to the elementary beam but the surface dose is increased. However, low and intermediate atomic number filters are excluded in broad clinical beams due to their excessive length and thus the advantages of broad high energy photon beams will be completely lost at these high energies.

However, broad photon beams could also be produced by scanning an elementary beam over a wide angular interval (cf Fig 2). The same mathematical technique as was used on the pencil electron beam (cf eq 2 and BRAHME & SVENSSON 1979) has been applied to the elementary photon beams. The result from such a calculation for a composite target of 1.5 mm tantalum and 70 mm aluminium also appears in Fig 12. The considerably improved deep penetration compared with the filtered beams and the elementary beam is striking. The small improvement in the build up region compared with the lead filtered beam and the degradation compared with the elementary beam should be expected. It is due to the increased electron contamination in broad photon

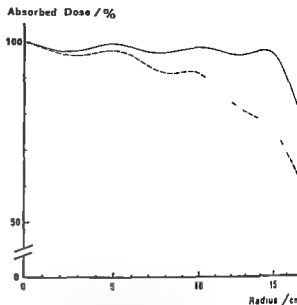


Fig 13 Lateral dose distribution obtained by 7x7 elementary photon beams of equal weight in a square grid (curve I). Curve II (—) obtained by doubling the weight at the edge.

beams and it is automatically obtained in proportion by the integration procedure. The reason for the increase over the elementary beam curve is that the contaminating electrons are spread over a larger area than the photons and thus their effect is obtained in the integration procedure.

When the scanned photon beam is used to produce broad beams, the grid or sieve technique may be of interest. In order to investigate the dose distribution across the scanned beam, a large number of crude beams were placed in a square grid pattern and their lateral dose distributions were added. The result of this procedure is given in Fig 13. The elementary beam used was that from a thick carbon target (cf Figs 10-11). Curve No 1 represents the dose variation across the major axis in a square field containing 7x7 grid points at a spacing of 5 cm. A considerable dose fall off near the edges of the beam is obtained due to the decreasing number of neighbouring beams near the edge of the field. Curve No 2 represents an almost perfect uniformity which is obtained by doubling the weight of the elementary beams at the edge giving a field size of 30 cm x 30 cm with a uniformity of ± 1.5 per cent.

From the high uniformity already at a grid spacing of 5 cm and the fairly narrow elementary beam from a thick carbon target, it is evident that very thin targets have to be used to get a strong grid effect in photon beams. This in turn would decrease the dose rate substantially, thus making the scanned beam technique less attractive for photon beams.

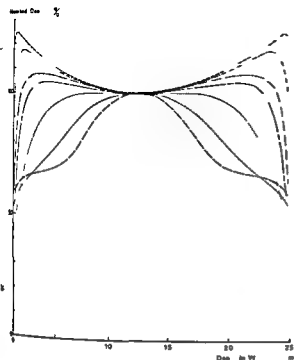


Fig 11 Central axis depth dose curves for parallel opposed broad photon and electron beams at a cross section of 25 cm. Considerably decreased integral dose is obtained with electrons for a central target volume. ^{60}Co (---) 4 (---) 10 (---) 25 (---) 50 (---) MeV respectively 50 (---) and 40 (---) MeV respectively

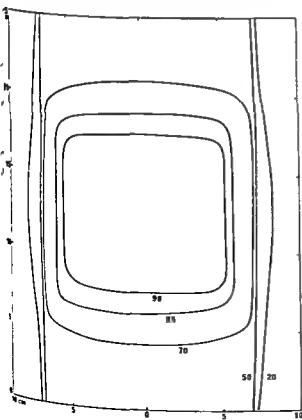


Fig 12

ever such narrow beams may still be of interest for use in the dynamic treatment of target volumes of complicated shapes without the need of moving heavy collimators

Parallel opposed beams

Given the capability of the racetrack microtron in delivering a wide range of radiation qualities in broad uniform beams of outstanding depth dose characteristics it is of interest to analyse how this may be used in treatment planning. For the sake of simplicity and due to its importance for deep seated tumours the plane parallel opposed beam situation has been chosen.

The high electron energies available make parallel opposed beams interesting for localized deep-seated tumours (OVADIA & UHLMANN 1960 ZATZ et coll 1961). In Fig 14 the central axis absorbed dose distributions for a 25 cm cross section are shown for a number of relevant beam qualities. Of special interest is that the parallel opposed electron beams give a much smaller integral dose than photon beams in the same geometry provided the target volume is smaller than about 10 to 15 cm. It is also possible to place the target volume eccentrically by using different electron energies or a combination of photons and electrons. In Fig 15 the isodose curves for parallel opposed 50 MeV electron beams for the case of a central target volume are shown. This distribution is based on the broad beam data in Fig 11. Due to the high electron energy used the penumbra at the target is still acceptable. Furthermore it should be pointed out that the high energy considerably reduces the effect of disturbing inhomogeneities that normally appear at lower energies. The isodose contours are thus not very different from the ideal dose distributions obtained with heavy charged particles like pions and protons with a dose to superficial tissues of only 60 to 70 per cent of the target dose.

In addition to the advantageous distribution of absorbed dose an additional biologic effect may be obtained if the beam is produced by scanning a narrow electron beam of very high dose rate and short duration (~ 50 ns). This will give a low mean dose rate to the target volume where many individual beams overlap due to the pear shape of each individual narrow electron beam (cf Fig 3). The sur-

Fig 12 Isodose contours (per cent) of 50 MeV parallel opposed electron beams. Due to high electron energy the penumbra is fairly narrow and the influence of inhomogeneities quite small

face dose will on the other hand be given by a single high dose rate pulse which could give increased survival according to the high dose rate effect observed by TODD et coll (1968). A possible explanation for this effect could be the reduced importance of oxygenation at pulse lengths which are comparable with the life time of most radiation induced radicals (10 ns). A smaller influence of the presence of oxygen should then be expected since the relative concentration of competing radiation induced radicals is increased. In addition this effect could also be used to explain the improved skin sparing observed by GRIEM et coll as their mechanically scanned narrow electron beam gave a much higher average dose rate to the superficial layers than to the tumour. A further possible advantage with such a scanned electron beam is the already discussed grid effect.

SUMMARY

The quality of broad high energy photon and electron beams can be improved considerably by the scanning of elementary narrow beams. Dose distributions produced in this way have advantages with respect to improved dose gradients and therapeutic ranges for electrons and lower surface doses, increased depth doses and half value depths for photons. The possibility of giving the whole therapeutic dose in a single pulse of short duration is discussed as a new method to protect superficial tissues.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Cancer Society and partly presented at the Sixth International Congress of Radiation Research in Tokyo 1979.

REFERENCES

- ALLIVERT M. Möglichkeiten und Perspektiven einer Ungleichmässigen Bestrahlung von malignen Geschwulsten. *Radiobiol Radiother* (Berl) 17 (1976) 223.
- ALCOUTURIER J, HUBER H et JAOVEN J. Systeme de transport du faisceau d'électrons dans le sagittaire. *Rev Tech Thomson CSF* 2 (1970) 655.
- BABIĆ B and SEDIČEK M. A method for stabilizing particle orbits in the racetrack microtron. *Nucl Instr Meth* 56 (1967) 170.
- BAUM G. Die verschiedenen Methoden der praktischen Durchführung der Siebstrahlung. *Strahlentherapie* 107 (1958) 397.
- BICKER J, GLUDIN F and KUTTIG H. Siebstrahlung mit ⁶⁰Co Gammastrahlen. *Strahlentherapie* 105 (1958) 2.
- BRAHME A. Investigations on the application of a microtron accelerator for radiation therapy. Thesis University of Stockholm 1975.
- Electron transport phenomena and absorbed dose distributions in therapeutic electron beams. 14th Int Congr Radiol Rio de Janeiro (1977) Abstract No S0348.
- and SVENSSON H. (a) Specification of electron beam quality from the central axis depth absorbed dose distribution. *Med Phys* 3 (1976) 95.
- (b) Methods of improving dose uniformity in high energy photon and electron beams. Digest of fourth International Conference on Medical Physics, Canada 32 (1976) 283.
- Electron beam quality parameters and absorbed dose distributions from therapy accelerators. Paper presented at the Int Symp on High Energy Electrons in Radiation Therapy, San Sebastian (1978).
- Radiation beam characteristics of a ²²MeV microtron. *Acta radiol Oncology* 18 (1979) 244.
- EPP E, R. WEISS H and SANTOMASSO A. The oxygen effect in bacterial cells irradiated with high intensity pulsed electrons. *Radiat Res* 34 (1968) 370.
- FROELICH H R and BRANNEN E. Four sector racetrack microtrons. *IEEE Trans Nucl Sci NS* 14 (1967) 746.
- GRIEM M L, SKAGGS L S, LANZ L L H and MALINSON F D. Experience in radiobiological dosimetry with high-dose rate electrons. In: *High energy radiation therapy dosimetry*. Ann N Y Acad Sci 161 (1969) 317.
- HARDER D. Durchgang schneller Elektronen durch dünne Materieschichten. Thesis University of Würzburg 1965.
- HSIEN C L and UHLMANN F M. Experimental evaluation of a 45 MeV medical linear accelerator. *Radiology* 67 (1956) 263.
- ICRU (International Commission of Radiation Units and Measurements) Report 21 (1972).
- JOLIS B. X ray sieve therapy in cancer. Lewis & Co London 1953.
- MACDONALD J C F and FROELICH H R. The racetrack microtron—its potential in radiation therapy. Proc III Conf on Application of Small Accelerators II p 314. Denton Texas (1974).
- NACP (Nordic Association of Clinical Physicists) Procedures in radiation therapy dosimetry with 5 to 40 MeV electrons and roentgen and gamma rays with maximum photon energies between 1 and 50 MeV. *Acta radiol Ther Phys Biol* 11 (1972) 603.
- Procedures in external radiation therapy dosimetry with electron and photon beams with maximum energies between 1 and 50 MeV. *Acta radiol Oncology* 19 (1980) 55.
- NBS (National Bureau of Standards). Neutrons from electron medical accelerators. Edited by H T Heatman and R Jacobs. NBS SP 554 Washington 1979.
- OVADIA J and UHLMANN E M. Isodose distribution and treatment planning with electrons of 20-15 MeV for deep seated tumors. *Amer J Roentgenol* 84 (1960) 754.
- PODORSKA E H, RAWLINSON J A, GLAVINOV M I

- JOHNS H E Design of X ray targets for high energy linear accelerators in radiotherapy *Amer J Roentgenol* 171 (1974) 873
- JOE J Beitrag zur Elektronentiefen Therapie mittels Röntgenbestrahlung *Strahlentherapie* 140 (1970) 156
- MEYER M LANZL L H NEWTON C M and SKAGGS S Computation of distribution of absorbed dose and absorbed dose rate from a scanning electron beam in radiotherapy 138 (1969) 651
- REY C B Cerebral radiation surgery with narrow gamma rays Physical experiments *Acta radiol Ther Phys et Biol* 13 (1974) 425
- REY C B Production of particle energies beyond 200 MeV *Rev sci Instr* 17 (1946) 6
- ROSENBERG J Quoted by Schiff
- ROSE P WINCHELL H S FEOLA J M and JONES G E Pulsed high-intensity roentgen rays *Acta radiol Ther Phys Biol* 7 (1968) 22
- VEKSLER V J A new method for acceleration of relativistic particles *Dokl Akad Nauk SSSR* 43 (1944) 329
- WERNHOLM O SEDLACEK M ROSANDER S and BABIK H A 50 MeV racetrack microtron *In* Summary of papers presented at the 4th All Union National Conference on Particle Accelerators p 117 Moscow 1974
- WILK H H and WILSON P B The racetrack microtron An electron accelerator for medium energies *In* Linear accelerators p 553 Edited by P M Lapostolle and A L Septier North Holland Amsterdam 1970
- ZATZ L M VON ESSEN C F and KAPLAN H S Radiation therapy with high energy electrons Part II Clinical experience 10 to 40 MeV *Radiology* 77 (1961) 925

SHORT-TERM INTRA-ARTERIAL MITOMYCIN C IN HEPATIC METASTASES

W. MATSSON, K. JONSSON, C. HELLEKANT and L. HALLSTEN

Untreated patients with hepatic metastases have a poor prognosis regardless of the site and nature of the primary tumour. The median survival of patients with liver metastases from colorectal carcinoma is 3 to 6 months (JAFÉ et coll. 1968, BENGMARK & HAFSTRÖM 1969, WOOD et coll. 1976) and from mammary carcinoma the median survival is 2 to 3 months (DEVITT 1971, MATSSON 1979). Further, if multiple organs are involved, the length of survival depends on the extent of the hepatic metastases (JAFÉ et coll.).

By using systemic chemotherapy or radiation therapy of hepatic metastases, no considerable increase of survival has been achieved, although varying temporarily symptomatic improvement has been observed (SHERMAN et coll. 1978).

Intra-arterial chemotherapy of the liver is attractive since both experimentally induced liver tumours (BREEDIS & YOUNG 1954) and flow measurements in humans (GELIN et coll. 1968) show that the malignancies receive almost all their blood supply from the hepatic arteries. Local administration of cytotoxic drugs intra-arterially was introduced by KLOPP et coll. in 1950. Thereafter, intra-arterial long-term infusions have been used in patients with hepatic metastases (ARIEL & PACK 1965, ANSFIELD et coll. 1974, OBERFIELD et coll. 1979, PETREK & MINTON 1979). The results have been modest. A median survival of the responders of about 6 to 10 months compared with 2 to 4 months in the non-responders. The rate of complications both to the technical procedure and to the treatment has been high. Further

more, the complicated procedure can permit only a minority of the patients to receive this form of treatment because of lack of resources and the high costs. However, intra-arterial combination chemotherapy (DOUGLAS 1979) combined intra-arterial chemotherapy and irradiation (FRIEDMAN et coll. 1979) and chemotherapy combined with starch microspheres (ARFORS et coll. 1979, AROENSEN et coll. 1979) have recently shown encouraging results.

Since the early sixties, angiography of various malignancies has been a routine procedure in this hospital in order to assess operability and prognosis. Japanese experience with short-term intra-arterial chemotherapy has shown promising results (KINAMI et coll. 1972, KINAMI & MIYAZAKI 1978). These facts initiated trials with a modified short-term intra-arterial Mitomycin C therapy in different tumours such as pulmonary carcinoma (HELLEKANT & SVANBERG 1978), gallbladder carcinoma (VON EYBEN et coll. 1980), head and neck carcinoma (MATSSON et coll. 1977, ANDERSSON et coll. 1979), pancreatic carcinoma (JONSSON et coll. 1980) and other malignancies including hepatic metastases.

Material and Methods

The material consisted of 30 patients admitted 1976 to 1979 to this Department of Oncology with

progressive biopsy proven hepatic metastases of evaluable/measurable extension where conventional treatment failed or had not been given (Table 1). Six patients were non-evaluable because of early death not related to the treatment (3 pulmonary carcinoma 2 colon carcinoma 1 melanoma). Six were males (4 colon carcinoma 1 leiomyosarcoma 1 oesophageal adenocarcinoma) 18 were females (5 colon carcinoma 7 mammary carcinoma 3 ovarian carcinoma 1 carcinoid 1 malignant lymphoma 1 unknown primary tumour). The median age was 57 years (34-74). The median free interval was 0 (0-30 months) in colon carcinoma 41 months (20-70 months) in mammary carcinoma 32 months (22-180 months) in ovarian carcinoma and 0 months (0-184 months) in unknown malignancies respectively.

Systemic chemotherapy of metastases had previously been given to 4/9 patients with colon carcinoma 6/7 with mammary carcinoma (all also endocrine resistant) 3 with ovarian carcinoma and 4/5 with unknown malignancies. After angiographic demonstration of the tumour and its vascularity the infusions were given as selectively as possible to the tumour feeding artery (arteries) i.e. the superior mesenteric or the coeliac trunk. In patients in whom the right lobe was supplied from an aberrant artery from the superior mesenteric artery or in whom the left lobe of the liver was supplied from the left gastric artery these vessels were selectively catheterized. All catheterizations were performed from either femoral artery with percutaneous technique. Angiography was carried out before each infusion. After each infusion the catheter was withdrawn. The patients were hospitalized for one day at the Department of Oncology during the treatment.

In 3 patients only one infusion was given. In 21 patients 2 to 9 infusions (median 3) were administered during a period of 2 weeks to 13 months. At each course the patients were given 10 to 15 mg Mitomycin C dissolved in 100 to 150 ml of physiologic saline. The infusion was given at a rate of 6 ml/min and the whole procedure was usually completed within 1½ hours.

Before each course all patients were examined physically. Karnofsky's index was assessed, complete blood counts, electrolytic status (potassium, sodium, creatinine, urate in serum), liver enzymes (ASAT, ALAT, ALP, GT), serum bilirubin were performed as well as radiography of the chest and angiography. Before every second or third course liver scintigraphy was performed in patients receiv-

Table 1

Short term intra arterial Mitomycin C treatment of hepatic metastases 1976 to 1979

Primary tumour	No of patients	Evaluable patients	Total No of courses	Median survival (months)
Colon	11	9	36	3
Breast	7	7	26	3
Ovary	3	3	6	2
Lung	3	0	-	-
Various	6	5	22	4
Total	30	24	90	3

Table 2

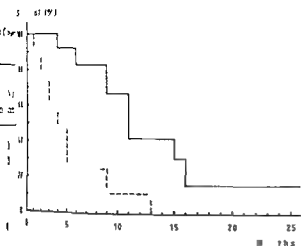
Short term intra arterial Mitomycin C treatment of hepatic metastases from colon carcinoma
OR=objective response NR=no response PD=progressive disease

Case	No of courses/dose (mg)	Results	Survival (months)
1	7/95	OR	9
2	4/60	OR	6
3	5/65	OR	9
4	3/45	NR	13
5	7/100	NR	3
6	3/30	PD	10
7	3/45	PD	7
8	1/10	PD	4
9	1/15	PD	-

Previously treated with systemic chemotherapy

ing only one course within 4 weeks of treatment. In patients ultrasound examination of the liver was carried out before and after treatment.

The results have been evaluated as follows: Complete remission, total normalization as demonstrated at angiography, liver scintigraphy, ultrasound of the liver, laboratory examinations and physical condition. Objective response, complete remission or subjective improvement at angiography or scintigraphy, ultrasound of the liver and normalization of liver enzymes ($\geq 30\%$ decrease of initial elevated values) and subjective improvement. No indication of progression as analysed with these methods was allowed. No response, unchanged condition or progression as recorded at angiography, scintigraphy,



Short term intra arterial Mitomycin C treatment in hepatic metastases Responders (—) Non responders (---) 0.001 < p < 0.01 (log rank test)

Table 3

Short term intra-arterial Mitomycin C treatment of hepatic metastases from mammary carcinoma CR=complete regression PR=partial regression NR=no response PD=progressive disease

Case	Previous chemo therapy	No of courses/ total dose (mg)	Results	Survival (months)
10	VACM	3/40	CR	15+
11	VAC	4/50	PR	11+
12	5-FU M ₁			
13	VACM	9/175	PR	15
14	VAC	4/40	PR	11
15	VACM	3/45	NR	5
16	CCNU L PAM	2/0	PD	2
17	VACM	1/10	PD	3

Table 4

Results of short term intra arterial Mitomycin C treatment of hepatic metastases

Primary tumour	No of objective regression/ No of patients
Colon	3/9
Breast	4/7
Ovary	2/3
Various	3/5
Total	12/24

ultrasound of the liver or increase of liver enzymes Survival was recorded from the start of the intra arterial chemotherapy

Results

An objective response was achieved in 12 of the 24 evaluable patients (Tables 2-4) The median survival was 11 months of the responders compared with 3 months for the non responders ($0.001 < p < 0.01$ log rank Figure) In 3 of the 9 patients with metastases from colon carcinoma an objective response occurred (Table 2) Among the non responders 2 patients had unchanged angiographic and scintigraphic appearance of the tumour as well as unchanged results of laboratory examinations at 4 and 7 months after beginning of treatment respectively Objective response was achieved in 4/7 patients with mammary carcinoma 2 patients are still alive in good condition without signs of hepatic metastases 11 and 15 months after beginning of the intra arterial treatment (Table 3)

Two of the 3 patients with ovarian carcinoma responded objectively and one of them is still alive at 28 months in a good state of health but with remaining tumour in the liver Each of the 3 patients with metastases from an unknown primary tumour leiomyosarcoma and adenocarcinoma of oesophagus respectively responded objectively No patient responded objectively after only one course of treatment while a symptomatic improvement was achieved in 9 patients Some regression of the tumour was registered in 8 of 12 responders after two courses although an objective response as defined did not occur until more courses were delivered (median 4 courses)

Side effects No complication of the technical procedure was encountered Subjectively the therapy was well tolerated Mild nausea easily treated with anti-emetics occurred in 10 patients during 36 courses (Table 5) Two patients had bronchopneumonia probably not treatment related (no leukopenia) The major problem was the cumulative thrombocytopenia which occurred at a median dose of 45 mg (10-85 mg) in 11 patients (Table 5) This thrombocytopenia was long standing and hampered further treatment in all these patients In 6 patients a normalization of platelets occurred within 3 months but in 5 the thrombocytopenia persisted for 3 to 11 months However no secondary bleedings occurred Otherwise no renal hepatic or other organ toxicity was observed

Complementary treatment after intra arterial Mitomycin C Two non responders of the 9 patients (Cases 4 and 5 Table 2) with hepatic metastases from colon carcinoma received 4 and 2 courses of combined intravenous 5 FU and Mitomycin C respectively also without objective regression. The 4 responders among patients with mammary carcinoma who all had metastases also in other organs received treatment from 6 weeks after the third intra arterial treatment with Mitomycin C and when the objective regression of this therapy was already documented. Two patients (Table 3 Case 11 with lytic bone metastases Case 13 with lytic bone metastases metastases of soft tissue and pleura) received weekly Adnamycin 20 mg. The other 2 patients (Case 10 with pleural and bone metastases Case 12 with pleural pulmonary and bone metastases) were treated with VACM regimen and high dose medroxyprogesterone acetate respectively.

Discussion

Short term intra arterial Mitomycin C chemotherapy of hepatic metastases is now a routine procedure with a negligible rate of technical complications. The treatment is subjectively well tolerated. Since an objective response was obtained in half of the patients with various malignancies it offers a measure of palliation which also has a positive cost benefit. The majority of patients needed only one day of hospital care at each course. The disadvantage of a high frequency of thrombocytopenia must however be considered. The thrombocytopenia is not easily explained although it is a well known side effect of Mitomycin C (CROOK & BRADY 1976). It might be due to the high concentration of the drug administered.

A low rate of platelet depression (not dose related) was observed in patients with mammary carcinoma treated with Mitomycin C administered intravenously (MATSSON *et coll.* 1980). In that regimen 10 mg Mitomycin C dissolved in 300 to 400 ml saline was infused over one hour every third week. The critical median level at which thrombocytopenia occurred in the present material was 45 mg. This is approximately the same as reported in a large survey from Japan (FRANK & OSTERBERG 1960).

The present results of short term intra arterial Mitomycin C treatment support the data from the original works in Japan (KINAMI *et coll.* KINAMI & MIYAZAKI) and compares with the results obtained

Table 5

Side effects following short term intra-arterial Mitomycin C treatment of hepatic metastases (per cent in parentheses)

	No of patients	No of courses
Nausea	10/4 (4*)	16/90 (40)
Thrombocytopenia	17/24 (71)	31/90 (37)
Infections	7/24 (8)	1/90 (1)

by long term infusions (ANSFIELD *et coll.* OBERFIELD *et coll.* PETREK & MINTON) a technique which has a considerably higher rate of side complications (OBERFIELD *et coll.* FORSBERG *et coll.* 1978) a high cost (PETREK & MINTON) and be offered only to a minority of patients because of the complexity of the procedure.

The median survival of patients with hepatic metastases from colo rectal carcinoma is shorter than patients without free interval regardless of treatment (OBERFIELD *et coll.* WOOD *et coll.*) compared with patients with a free interval. In the present material 5 of the 9 patients with metastases from colo rectal carcinoma had no free interval. Three of them responded objectively and survived more than 6 months which is about double the expected survival without treatment. However, the results of treatment in hepatic metastases from colo rectal carcinoma is still unsatisfactory. Because of the encouraging results obtained by combined measures (irradiation and intra arterial chemotherapy - FRIEDMAN *et coll.*) and by enhancing the effect of cytostatic drugs via a decreased circulation (ARONSON *et coll.*) the intention is now to combine the short term intra arterial chemotherapy with starch microspheres and external irradiation.

The effect of intra arterial short term Mitomycin C therapy in hepatic metastases from mammary carcinoma and ovarian carcinoma resistant to systemic chemotherapy or endocrine treatment has been encouraging. As the method is simple and well tolerated this treatment is now recommended as supplement to the intravenous chemotherapy.

SUMMARY

Short term intra arterial Mitomycin C treatment of hepatic metastases from various malignancies was performed.

in 14 evaluable patients. An objective regression was obtained in 12 patients. The median survival of responders was 11 months compared with 3 months in non-responders. No complication of the technical procedure occurred and the treatment was well tolerated. The only side effect of clinical importance was thrombocytopenia which occurred at a median dose of 45 mg of Mitomycin C.

Request for reprints: Dr W Mattsson, Department of Oncology and Radiation Therapy, Allmänna Sjukhuset, S-21401 Malmö, Sweden.

REFERENCES

- ANDERSSON T, ANDREASSON L, BJÖRALUNDA B, BRISMAR J, ELLNER Å, ENEROTH C M, HELLEKANT C, LUNDBERG T and MATTSSON W. Intra arterial chemotherapy of malignant head and neck tumours with a superselective angiographic technique. *Acta otolaryng* (1979) Suppl No 360 p 167.
- ASTFIELD F J, RAMIREZ G, DAVIES JR H L, WIRTA NENG W, JOHNSON R O, BRYAN G T, MANALO F B, BORDEN E C, DAVIES T E and ESMAILI M E. Further clinical studies with intrahepatic arterial infusion with 5 Fluorouracil. *Cancer* 36 (1975) 2413.
- BERGSTRÖM L, ARONSEN K, ROTHMAN U and REGELOU W. The use of amylose biodegradable starch microspheres as a tumour infarcting and delivery system for chemotherapy. Intermittent hepatic arterial occlusion and the delivery of regionalized chemotherapy to hepatic metastases. *Amer Soc Clin Oncol* 20 (1979) 370.
- BRIEL I M and PACK G T. Intraarterial chemotherapy for cancer metastatic to liver. *Arch Surg* 91 (1965) 851.
- BROSEY K F, HELLEKANT C, HOLMBERG J, ROTHMAN U and FEDER H. Controlled blocking of hepatic artery flow with enzymatically degradable microspheres combined with oncolytic drugs. *Europ Surg Res* 11 (1979) 99.
- ENGELBARK S and HAFSTRÖM L. The natural history of primary and secondary malignant tumours of the liver. *Cancer* 23 (1969) 198.
- ESKES C and YOUNG G. The blood supply of neoplasms in the liver. *Amer J Pathol* 30 (1954) 969.
- FOOKE S T and BRADNER W T. Mitomycin C. A review. *Cancer Treat Rev* 3 (1976) 121.
- FRITZ T J E. The enigmatic behaviour of breast carcinoma. *Cancer* 27 (1971) 12.
- FRYGLAS C C. Improved survival in liver metastases from colorectal carcinoma following periodic arterial infusions with Mitomycin C 5 Fluorouracil. *Adriamycin Velban and Vincristine. Amer Soc Clin Oncol* 10 (1979) 431.
- VON EYBEN F, HELLEKANT C, MATTSSON W, LUNDAQUIST U and JONSSON K. Mitomycin C in advanced gallbladder carcinoma. *Acta radiol Oncology* 19 (1980) 81.
- FRYBERG L, HAFSTRÖM I O, LUNDAQUIST A and SUNDQVIST K. Arterial changes during treatment with intra hepatic arterial fusion of 5 Fluorouracil. *Radiology* 126 (1978) 49.
- FRANK W and OSTERBERG A E. Mitomycin C (NSC 26980). An evaluation of the Japanese reports. *Cancer Chemother Rep* 9 (1960) 114.
- FRIEDMAN M, CASSIDY M, LEVINE M, PHILLIPS TH, SPIVACK S and RESSER K J. Combined modality therapy of hepatic metastases. *Cancer* 44 (1979) 906.
- GELIN L E, LEWIS D H and NILSSON L. Liver blood flow through metastatic tumour nodules. *Acta hepato splenol* 15 (1968) 21.
- HELLEKANT C and SVANBERG L. Bronchial arterial infusion of Mitomycin C in advanced bronchogenic carcinoma. *Acta radiol Oncology* 17 (1978) 449.
- JAFFE B M, DONEGAN W L, WATSON F and SPRATT J S. Factors influencing survival in patients with untreated hepatic metastases. *Surg Gynec Obstet* 127 (1968) 1.
- JONSSON K, MATTSSON W, HELLEKANT C and VON EYBEN F. Intraarterial short term chemotherapy of inoperable pancreatic cancer. Submitted for publication 1980.
- KINAMI Y and MIYAZAKI I. The superselective and the selective one short methods for treating inoperable cancer of the liver. *Cancer* 41 (1978) 1720.
- HIROSE M and TAKAMUTU O. One short administration of antineoplastic agents in massive doses via the coeliac artery for inoperable cancer of the liver. *Jap J Surg* 2 (1972) 122.
- KLOPP C T, ALFORD T C, BATEMAN J, BERRY G N and WINSHIP T. Fractionating intraarterial cancer chemotherapy with methyl bisamine hydrochloride. A preliminary report. *Ann Surg* 132 (1950) 811.
- MATTSSON W. Endocrine treatment and combination chemotherapy in metastatic postmenopausal breast cancer. Thesis 1979.
- HELLEKANT C and ANDREASSON L. Combination chemotherapy of advanced squamous carcinoma of the head and neck. *Acta radiol Ther Phys Biol* 16 (1977) 385.
- VON EYBEN F and HALLSTEN L. A phase II study of combined Mitomycin C and 5 Fluorouracil in advanced breast cancer. Submitted for publication 1980.
- OBERFELD R A, McCAFFREY J A, POLIO J, CLOUSE M F and HAMILTON TH. Prolonged and continuous percutaneous intraarterial hepatic infusion chemotherapy in advanced metastatic liver adenocarcinoma from colo rectal primary. *Cancer* 44 (1979) 414.
- PETREK J A and MINTON J P. Treatment of hepatic metastases by percutaneous hepatic arterial infusion. *Cancer* 43 (1979) 2182.
- SHERMAN D M, WEICHSELBAUM R, ORDER S E, CLOUD L, TREY CH and PIRO A J. Palliation of hepatic metastases. *Cancer* 41 (1978) 2013.
- WOOD C B, GILLIS C R and BLUMGART L H. A retrospective study of the natural history of patients with liver metastases from colo rectal cancer. *Clin Oncol* 2 (1976) 285.

FROM THE DEPARTMENT OF GENERAL ONCOLOGY RADIUMHEMMET KAROLINSKA SJUKHUSET S 10401
STOCKHOLM AB KABI DIAGNOSTICA S 11287 STOCKHOLM AND THE DEPARTMENT OF HOSPITAL PHYSICS
SÖDERSJUKHUSET S 10064 STOCKHOLM SWEDEN

³²P PYROPHOSPHATE IN THE TREATMENT OF PERSISTENT METASTATIC BONE PAIN

■ WERNER C ISACSON G LUNDELL C LONNBECK and B SÖDERBORG

Among the beta emitting radiopharmaceuticals used in the alleviation of pain caused by bone metastases probably the one in most common use is ³²P orthophosphate given either intravenously or orally (FRIEDEL & STORAASLI 1950 MAXFIELD JR et al 1958 MILLER 1974). However the dose required for adequate palliation frequently causes such severe bone marrow depression as to prevent its adoption for routine use.

As the beta energy and physical half life of ³²P are suitable other compounds of this nuclide were sought with a less marked tendency to cause bone marrow depression than the orthophosphate while being in least as effective as a palliative. The pyrophosphate was considered to merit examination for this purpose. Furthermore it was hoped that in the metabolism of the ³²P pyrophosphate the nuclide metabolites would prove to be incorporated in the neighbouring tumour cells with potential tumoricidal effect.

Of intravenously administered ³²P pyrophosphate 40 per cent is known to be excreted by the kidneys. In addition in preliminary in vivo experiments ³²P pyrophosphate uptake was at least 5 times greater in metastatic deposits in bone than in normal bone (SÖDERBORG & WERNER unpublished results). The radiation dose from 37 MBq (1 mCi) of ³²P pyrophosphate administered intravenously to adults was calculated to be of the order of 4.0 Gy in metastatic regions against 0.8 Gy in normal bone in

which case the dose received by the bone marrow would be much lower.

It was accordingly considered of interest to examine the haematologic and palliative effects of therapeutic amounts of ³²P pyrophosphate in patients with persistent metastatic bone pain. (Permission was obtained from the Ethic Committee of Karolinska Sjukhuset and the informed consent of each patient was obtained.)

Materials and Methods

³²P Na₂P₂O₇ was prepared by mixing solutions of H₃³²PO₄ (0.1 ml 37 GBq (1 Ci)/ml) and Na₂HPO₄ (120 mg 30 mg/ml). The mixture was evaporated to dryness and heated at 400°C for 2 hours. The residue was dissolved in sterile water and then sterile filtered. The solution was divided into single doses (370–555 MBq) which were freeze dried. The product then being stable for up to 3 weeks as determined by paper chromatography. For reconstitution sterile saline was used (0.9 mg/ml).

Eight patients (7 females 1 male) with persistent pain due to bone metastases from mammary carcinoma were given ³²P pyrophosphate. For at least 6 months before the administration none of the patients had advanced depression of the haematologic system or had received radiation therapy or

Table

Least peripheral blood counts before and after administration of ^{32}P pyrophosphate. Effect on pain and duration of relief

Case No	Hb (g \times l)		Leukocytes (10 9 \times l)		Thrombocytes (10 9 \times l)		Effect	Duration (weeks)
	Before	After	Before	After	Before	After		
1	126	91	7.0	0.2	200	60	++	10
2	118	81	3.7	0.3	245	3.6	0	-
3	114	80	8.2	1.6	340	36	+	4
	126	92	7.1	4.4	149	70	+	6
4	133	130	6.1	7.0	255	250	+	5
5	106	80	4.7	1.8	196	16	++	10
	76	59	8.2	0.8	37	8	++	10
6	79	104	5.1	2.4	94	28	+	4
7	100	98	4.6	1.1	136	18	++	10
8	127	103	9.3	4.7	289	189	++	9

chemotherapy 444 MBq of ^{32}P pyrophosphate was given intravenously to the first 3 patients and 370 MBq to the other 5 once to 6 of the patients and on two occasions to the other 2.

Peripheral platelet and leukocyte counts, determination of the haemoglobin concentration and liver and renal function tests were performed before administration of the nuclide and afterwards at intervals of one week for the first 6 weeks and then in alternate weeks. Bone radiography and bone scintigraphy using $^{99}\text{Tc}^m$ pyrophosphate were performed immediately before administration and again after 3 months.

The effect of the therapy on pain was evaluated subjectively according to a three grade scale: increased pain or no reduction (0), some alleviation (+) and complete alleviation with no further reliance on analgesic drugs (++)

Results

After the ^{32}P pyrophosphate therapy all but one of the patients reported some relief of pain (Table). It occurred first in the second week after the injection and lasted for 1 to 4 months. The alleviation was greatest for patients with small metastases and less marked for those with collapsed vertebrae or extensive bone destruction.

In all but one of the patients the peripheral blood cell elements depressed. For white cells and platelets this decrease began in the third week after the therapy, the nadir occurred at 4 to 5 weeks and normalization was usually recorded at 6 weeks. The

erythrocyte counts were still slightly depressed after 2 to 3 months.

After the therapy the serum levels of alkaline phosphatase usually tended to be slightly reduced. Other biochemical tests of the hepatic renal and coagulation systems were unaffected. In one case a elevated serum calcium level was normalized following treatment.

In 6 patients no changes were found at bone scintigraphy or radiography up to 3 months after treatment. In the other 2 patients lytic areas were calcified.

In 2 patients deep venous thrombosis appeared a leg 3 weeks after administration of the nuclide. In both of them anticoagulant therapy was successful.

Discussion

The various organic and inorganic ^{32}P compounds that have been tested in therapeutic trials in man have proved to be roughly equally effective in alleviating pain. They have also produced similar side effects arising mainly from depression of haematopoietic tissues (KAPLAN et coll 1960; POTSAID et coll 1978).

In the present series all but one of the 8 patients experienced less pain, a proportion that is similar to those reported after use of other ^{32}P compounds with or without pretreatment with testosterone or other hormones (MAXFIELD JR et coll 1971). It is notable that the only patient not experiencing any pain reduction had essentially normal values of serum alkaline phosphatases despite generalized

dissemination of the disease and an elevated uptake of $^{99\text{m}}\text{Tc}$ pyrophosphate in metastatic bone as demonstrated at scintigraphy

In all but one of the patients haematologic depression occurred serious in one case but not lethal. The platelet count seemed to be most susceptible with a nadir at 3 to 4 weeks after the administration of ^{32}P . At the same time also a profound but less marked depression of the leukocyte count occurred.

In the comparison of various methods for relieving pain the selection of the patients is obviously important. Patients receiving nuclides early in the course of the disease will probably experience a greater relief than those with extensive bone destruction. Irrespective of any such selective factor in the present series the side effects were clearly too marked for ^{32}P pyrophosphate to be recommended for routine palliative treatment of persistent pain due to bone metastases. From the observed side effects it would appear that the pyrophosphate molecule is broken down and that ^{32}P is then probably taken up by the bone marrow cells.

SUMMARY

Eight patients with persistent pain due to disseminated bone metastases from mammary carcinoma were given

about 370 MBq (10 mCi) of ^{32}P pyrophosphate on 10 occasions. All but one of the patients experienced alleviation of pain lasting 1 to 4 months. The side effects which derived mainly from haematopoietic tissue prevent the routine use of this compound.

Request for reprints: Dr Björn Werner, Department of General Oncology, Radiumhemmet, Karolinska Sjukhuset, S-10401 Stockholm, Sweden.

REFERENCES

- FRIEDEL H L and STORAASLI J P. The use of radioactive phosphorus in the treatment of carcinoma of the breast with widespread metastases to bone. *Amer J Roentgenol* 64 (1950) 559.
- KAPLAN E, FELS I G, KOTLOWSKI H R, GRECO J and WALSH W S. Therapy of carcinoma of the prostate metastatic to bone with P^{32} labeled condensed phosphate. *J nucl Med* 1 (1960) 1.
- MAXFIELD JR J R, MAXFIELD J G S and MAXFIELD W S. The use of radioactive phosphorus and testosterone in metastatic bone lesions from breast and prostate. *Sth med J* 51 (1958) 320.
- MILLER A. Radiophosphorous (P^{32}) treatment in carcinoma of the breast and prostate. A report of 39 cases. *J Amer osteopath Ass* 74 (1974) 217.
- POTSARD M S, IRWIN JR R J, CASTRONOVO F P, PROUT JR G R, HARVEY W J, FRANCIS M H, TOFE A J and ZAMENHOF E G. ^{32}P diphosphonate dose determination in patients with bone metastases from prostatic carcinoma. *J nucl Med* 19 (1978) 98.

FROM THE DEPARTMENTS OF NUCLEAR MEDICINE AND OF ONCOLOGY AND RADIATION THERAPY THE
RADIIUM CENTRE AARHUS KOMMUNEHOSPITAL AND UNIVERSITY OF AARHUS DK-8000 AARHUS DENMARK

SERUM CALCITONIN IN PATIENTS WITH OSTEOLYTIC AND OSTEOSCLEROTIC METASTASES FROM MAMMARY CARCINOMA

H E NIELSEN and C CH GADEBERG

Increased serum immunoreactive calcitonin (S iCT) concentrations have been found in patients with mammary carcinoma (COOMBES et coll 1976, W. RAUD et coll 1977, OLSEN et coll 1978). COOMBES et coll found elevated S iCT in 23 of 28 patients with breast carcinoma and metastatic disease whereas only one of 13 with localized disease had increased levels and OLSEN et coll elevated S iCT in patients with osteolytic bone metastases as compared with patients with local tumour. However 7 patients with osteosclerotic metastases had very low S iCT concentrations.

In contrast to these authors ROUSSEAU et coll (1978) found marked hypercalcitonemia in a patient with osteosclerotic myeloma and suggested that osteolytic bone lesions could be induced by ectopic secretion of calcitonin by tumour cells. Therefore it was considered of interest to investigate whether S iCT differed between patients with mammary carcinoma and osteolytic and osteosclerotic bone lesions and furthermore to compare biochemical and clinical values in these two patient groups.

Material and Methods

The series consisted of 27 women with microscopically proven mammary carcinoma and with radiologically proven bone metastases. 19 had osteolytic and 8 osteosclerotic metastases. The mean age of the former group was 57.3 years (range 38–79) and of the latter 60.8 years (range 45–79). None

of the patients received drug therapy or irradiation at the time of the present investigation. All patients had normal renal function evaluated by serum creatinine.

Serum concentrations of calcium, phosphorus, protein and calcitonin (S iCT) were measured after fasting overnight. Serum calcium was corrected for individual variation in serum protein concentration (PEDERSEN 1973) and calculated as the calcium concentration corresponding to a protein level of 70 g/l (S calcium corr). S iCT was measured by a radioimmunoassay as described previously (NIELSEN et coll 1979, NIELSEN & OLSEN 1979) using a commercial antibody to synthetic human calcitonin (Calbiochem, USA) and synthetic human monomer calcitonin (Ciba, Switzerland) for standards and iodination. The detection limit was 20 pg/ml. Normal range was 0 to 120 pg/ml and detectable values could be measured in 85 per cent of normal males and 59 per cent of normal females. The intraassay coefficient of variation was below 3 per cent at a serum concentration of 350 pg/ml and 14 per cent at a serum concentration of 30 pg/ml. The interassay variation coefficient was 14 per cent at a S iCT concentration of 140 pg/ml.

A group of 13 normal women with a mean age of 47.4 years (range 37–57) was used as control group for the S iCT values.

Statistical evaluation: Spearman's rank correlation

tion test was used for correlation analysis. The Mann Whitney U test was used for comparison of group means.

Results

A significantly higher S-CT concentration was found in patients with osteolytic metastases as compared with patients with osteosclerotic ($p < 0.01$, Figure) and with normal controls ($p < 0.01$). No difference was found in S-CT between patients with osteosclerotic metastases and normals. In the osteolytic group 11 of 19 patients appeared with S-CT values higher than the maximum value in the control group whereas in the osteosclerotic group all S-CT values were normal.

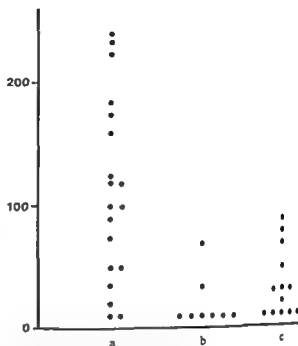
One patient in the osteolytic group had hypercalcemia and 9 patients elevated S-alkaline phosphatase. None of the patients in the osteosclerotic group had hypercalcemia whereas 6 had increased S-alkaline phosphatase. No significant difference was found in the values of S-calcium, S-phosphorus or S-alkaline phosphatase between the two groups (Table) nor any difference in the histologic classification of the tumour or in the localization of the bone metastases.

Discussion

Increased S-CT was found in 11 of 19 patients with osteolytic bone metastases in contrast to normal S-CT concentration in all 8 patients with osteosclerotic metastases. Previously a high frequency of elevated S-CT concentration was found in patients with metastatic disease from mammary carcinoma (COOMBS *et al.* 1976, OLSEN *et al.*).

RASMUSSEN *et al.* (1978) compared S-CT in

S-CT pg/ml



Serum calcitonin in patients with mammary carcinoma: a) 19 patients with osteolytic metastases, b) 8 with osteosclerotic metastases, c) Normal controls.

patients with osteolytic and osteosclerotic metastases. Normal or undetectable S-CT was found in all but one of 26 patients, and no difference was demonstrated between patients with osteolytic and osteosclerotic metastases. The finding of normal or undetectable S-CT values in most patients with osteolytic bone metastases is in contrast to most other reports on patients with breast carcinoma (COOMBS *et al.* 1976, MILHAUD *et al.* 1977, OLSEN *et al.*). It might be due to that the patients in this series of these authors were treated with pre-

Table

Biochemical values (mean range) in patients with mammary carcinoma and osteolytic and osteosclerotic metastases

Metastases	S-calcium corr (mg/100 ml)	S-phosphorus (mg/100 ml)	S-alkaline phosphatase (units)	S-CT (pg/ml)
Osteolytic (n=19)	9.74 (9.1-11.7)	3.6 (2.6-4.7)	476 (101-300)	11 (10-40)
Osteosclerotic (n=8)	9.75 (9.1-10.6)	3.4 (2.5-4.2)	283 (149-590)	21 (10-70)
P	NS	NS	NS	<0.01

more anti-oestrogen and cytostatics during the analysis period

Increased S-CT in patients with malignant disease other than medullary thyroid carcinoma has been explained by an ectopic CT production from the tumour (COOMBES et coll 1974 SILVA et coll 1974) or by a physiologically increased CT secretion from the thyroid (SILVA et coll 1975) to prevent osteolytic processes in the bones. In patients with pulmonary carcinoma both mechanisms have been demonstrated (SILVA et coll 1974 1975). In vitro experiments with lung and breast carcinoma tissue (COOMBES et coll 1976) both types of tumour have been found to secrete CT. The present finding of increased S-CT in patients with breast carcinoma osteolytic but not in those with osteosclerotic metastases support the view that increased S-CT may be due to a physiologically increased CT secretion to prevent osteolysis rather than an ectopic production of CT.

SUMMARY

Serum concentrations of calcitonin calcium phosphorus and alkaline phosphatase were measured in 19 patients with osteolytic metastases and in 8 patients with osteosclerotic metastases from mammary carcinoma. A significantly higher mean S-CT concentration was found in patients with osteolytic metastases as compared with those with osteosclerotic. No significant difference was found in S-calcium S-phosphorus or alkaline phosphatase between the two groups.

ACKNOWLEDGEMENTS

The investigation was supported by the Danish Medical Research Council (No 512 10195). Synthetic human calcitonin was kindly donated by Ciba, Switzerland.

REFERENCES

- COOMBES R C GREENBERG P B and HILLYARD C Plasma immunoreactive calcitonin in patients with non thyroid tumours. *Lancet* I (1974) 1080
- ELLISON M L EASTY G C HILLYARD C J JAMES R GALANTE L GIGIS S HEYWOOD L MACINTYRE I and NEVILLE M The ectopic secretion of calcitonin by lung and breast carcinomas. *Clin Endocr* (1976) Suppl No 5 p 387
- MILHAUD G CALMETTES C JULLIENNE A RIBEIRO F and MOUKHTAR M S Calcitonin. In *Proc of International Congress of Endocrinology*, Hamburg 1976 p 430. Edited by V H T James. Excerpta Medica Amsterdam Oxford 1977
- NIELSEN H E and OLSEN K J Serum calcitonin after renal transplantation. *Acta med scand* 205 (1979) 619
- CHRISTENSEN C K and OLSEN K J Serum calcitonin in patients with chronic renal disease. *Acta med scand* 205 (1979) 615
- OLSEN K J GADEBERG C NIELSEN H E and JOHANNSEN A Increased serum calcitonin in patients with mammary carcinoma. *Acta radiol Oncology* 17 (1978) 263
- PEDERSEN K O Calcium in human serum. Biochemical and clinical aspects. Thesis University of Aarhus 1973
- RASMUSSEN B ROESDAHL K and LINDGREEN P Parathyroid hormone and calcitonin in serum of patients with mammary carcinoma. *Acta radiol Oncology* 17 (1978) 269
- ROUSSEAU J J FRANCK G GRISAR T REZNIK M HEYVEN G and SALMON J Osteosclerotic myeloma with polyneuropathy and ectopic secretion of calcitonin. *Europ J Cancer* 14 (1978) 133
- SILVA O L BECKER K L PRINACK A DOPPMAN J and SNIDER R H Ectopic secretion of calcitonin by oatcell carcinoma. *New Engl J Med* 290 (1974) 1122
- — — — — Hypercalcitonemia in bronchogenic cancer. Evidence of thyroid origin of the hormone. *J Amer med Ass* 234 (1975) 183

PALLIATIVE IRRADIATION OF BRAIN METASTASES

C I C TURALBA A M EL MAHDI and W J PEEPLES

Materials and Methods

The treatment of brain metastases is discussed periodically as the search continues for the best means of palliation considering the patient's survival and quality of life, hospitalization period, treatment cost, convenience to physician and patients. As POSNER & SHAPIRO (1975) have stated, it is a frustrating problem for the following reasons: the tumors are difficult to control, the evaluation of survival time is complicated by the fact that usually the metastases are widespread and many who respond to treatment of the metastatic brain tumor succumb to other complications of their systemic malignancy.

The incidence of brain metastases varies from 2.2 to 35 per cent depending on the institution and the material examined. Before CHAO et al (1954) reported on the irradiation management of brain metastases, palliation was achieved with surgery in selected cases (STORTEBECKER 1954). Since then, palliation therapy has become the palliative treatment of choice as it achieves the same results as surgery without the high mortality rate observed in the latter. The dose of radiation has varied from 15 Gy single dose (HINDO et al 1970, JACZ & LARSEN 1974, SHEHATA et al 1974, YOUNG et al 1974) to 55 Gy in 5 1/2 weeks in a few cases (ORDER et al 1968), although the commonly suggested dose is 30 Gy in 2 weeks (MONTAGUE 1973, POSNER 1974). The best palliative dose has remained unsettled. Over the last 3 years in this institution, 30 Gy in 2 weeks has been the most commonly used dose. It appeared to provide adequate palliation in most cases; however, a recent wave of recurrences prompted us to review the results of this treatment schedule.

The records of 121 patients with brain metastases evaluated for palliative irradiation from January 1976 to December 1978 were reviewed. There were 70 males and 51 females ranging in age from 34 to 81 years, with both the median and mean age at 59.89. They were white 32, black 6.

The lung was the most common site of the primary tumor (81/121, 66.9%). Of these lung primaries were 36 (44.4%) well differentiated squamous cell carcinoma, 15 (18.5%) poorly differentiated squamous cell carcinoma, 21 (26%) adenocarcinoma and 9 (11.1%) oat cell carcinoma (Table 1). The breast was a distant second in frequency (14/121, 11.6%). The primary tumor was unknown in 8 cases. Fewer cases had other primary tumors (Table 1). The case with multiple myeloma was not a typical parenchymal lesion but was actually an extension of a large skull lesion into the brain, causing the same signs and symptoms. This lesion was debulked at craniotomy and the patient was referred for postoperative irradiation.

The presenting signs and symptoms appear in Table 2. As in several other series (BAKER 1942, LESSE & NETSKY 1954, MACGEE 1971, NISCE et al 1971, STORTEBECKER), hemiparesis was the most common sign, although this occurred in only 34 per cent. Headache was the most common symptom.

The diagnostic procedure used was scintigraphy with ^{99m}Tc in 56 patients (46%), computed tomography in 48 (40%) and both procedures in 13 (11%). In 2 patients, brain metastases were documented at angiography while in one case the EEG result was

felt to be compatible with the diagnosis. In one case the procedure was not specified.

The distribution of patients according to the treatment modality used is given in Table 3. No irradiation was given to 8 patients, 7 of these being in too bad condition due to their systemic disease to warrant even palliative irradiation. In the eighth patient a craniotomy was performed with excision of a solitary lesion and no post-operative radiation therapy was given. Craniotomy was performed in 10 cases either to establish the histologic diagnosis or to rule out a primary brain tumor. The operation was followed by irradiation. Another patient had hydrocephalus secondary to an obstructive metastatic lesion in the fourth ventricle and a shunt was inserted before irradiation. Irradiation alone was given to 101 patients, 90 of these received 30 Gy in 2 weeks. Seven patients who received 15 Gy or less actually did not finish the radiation therapy due to progression of their systemic disease.

All the irradiated patients were treated with a 4 MV irradiator except for one patient who was treated with a ^{60}Co . All patients received whole brain irradiation using lateral opposing fields. The usual dose rate was 3 Gy.

Systemic chemotherapy before and occasionally during their irradiation course was administered to 4 of the 109 patients. This was not felt to have influenced the results of irradiation and was not included in the analysis.

Based on the initial history and physical examination the patients were classified as to neurologic functional status using the modification made by MONTANA *et al.* (1972) of the classification of OLFER *et al.* (Table 4). Evaluation of the functional status was made at the end of the treatment. Patients were followed at 1 month and 3 months after treatment then at 6-month intervals thereafter.

Results

The different diagnostic procedures revealed solitary lesions in 65 cases (53.7%). This was near the higher range of the 10 to 64 per cent incidence of solitary lesions reported by other authors (ASK UP MARK 1956, BAKER, CHASON *et al.* 1963, LESSE & NETSKY, RICHARDS & MCKISSOCK 1963). Thirty-eight (58.5%) of these 65 solitary lesions were located in the frontal and temporal regions. Both hemispheres were almost equally involved. Multiple lesions were found in 54 patients (44.6%). In 2

Table 1
Sites and distribution of primary tumors

Site	No.	Per cent
Lung	81	66
Squamous cell carcinoma well differentiated	36	
Squamous cell carcinoma poorly differentiated (including large cell)	15	
Adenocarcinoma	21	
Oat cell	9	
Breast	14	11
Unknown primary tumor	8	6
Adenocarcinoma	4	
Undifferentiated carcinoma	2	
No histology	2	
Melanoma	6	5
Colon	6	5
Kidney	3	2
Urinary bladder	1	
Tongue	1	
Multiple myeloma	1	
Total	171	

Intracerebral extension of skull metastasis

Table 2
Presenting signs and symptoms

	No.	Per cent
Hemiparesis	41	34
Headache	34	8
Aphasia	11	9
Visual impairment	10	8
Seizure	7	6
Ataxia	6	5
Confusion	6	5
Syncope	5	4
Vertigo	5	4
Personality change	4	3
Nausea and vomiting	3	2
Coma	2	
Hypopituitarism	1	
Memory loss	1	
Numbness of face	1	

cases brain scintigraphy was normal but all findings were highly suggestive of brain metastasis and the primary tumor was not controlled therefore they were treated palliatively. In 53 of the 171 cases

Table 3

Distribution as to treatment

	No of cases
No treatment	7
Cranotomy only	1
Surgery + radiation therapy	12
Cranotomy + 30 Gy/7 weeks	10
Cranotomy + 34 Gy/7 1/2 weeks	1
Shunt + 30 Gy/7 weeks	1
Radiation therapy only	101
45 Gy/4 weeks	2
40 Gy/3 weeks	1
30 Gy/7 weeks	90
0 Gy/1 week	1
15 Gy/1 week or less	7
Total	171

reviewed brain metastases were diagnosed at the same time as the primary lesion. 50 of these had pulmonary carcinoma.

The distribution of patients as to their response to treatment based on improvement of the functional status appears in Table 5. Sixty per cent of the patients improved. In certain Class II and III patients with several signs and symptoms amelioration of some symptoms occurred but little or no improvement of their other neurologic deficits. Those patients were kept in their pretreatment classification. A few of these patients improved further during the course of their follow up. Interestingly, the tabulation of the functional status shows a greater number of the present patients in Class I compared with those of MONTANA et coll, ORDER et coll and SHEHATA et coll. Most of the patients in this class had symptoms of headache, aphasia, visual impairment and other minor symptoms which were temporarily relieved by steroids. Therefore, their neurologic examination was unremarkable at the time of evaluation for radiation therapy.

Only seven of the irradiated patients failed to finish their treatments. One of these patients belonged to Class II, 3 to Class III, and 3 to Class IV before treatment. Six of these 7 patients had progressive systemic disease at the time of whole brain irradiation which caused their rapid deterioration. All of these 6 cases had primary pulmonary carcinoma.

The overall recurrence rate was 17.7 per cent (Table 6) with pulmonary lesions comprising more than half of these recurrences. The overall rate of retreatment was 8.8 per cent (10/113). Considering the recurrences in relation to their representative number in the patient population, only 14.8 per cent of the metastases from the pulmonary tumor recurred compared with 42.8 per cent of those from the breast tumor. Only half of the recurrences from these respective sites were retreated. In the other half, the metastases were noted in the terminal stages of the disease. The average time between the initial brain irradiation and retreatment was 10.8 months for the metastases from lung tumors and 5.3 months for those from breast tumors. The range for the whole group was 2.5 to 15 months. Of these recurrences, 7 developed within 3 months from first treatment. Four of these 7 cases lived for a few more weeks while the other 3 died of their primary disease around the same time that their brain recurrence was noted. The reason for the longer interval noted in

Table 4

Functional classification. Adapted from ORDER et coll. *Radiation Therapy* (1958) 149; modification by MONTANA et coll. *Cancer* 29 (1972) 1477. Reprinted with permission.

Class	Definition
I	Neurologic findings minor or not present
II	Neurologic findings present but not a major factor; no hospitalization required
III	Major neurologic findings present; hospitalization required
IV	Profound neurologic deficit; hospitalization essential

Table 5

Response to treatment based on neurologic function improvement

Before treatment	Neurologic function status			
	After treatment			
	I	II	III	IV
I				
II	31	7	1	
III	18	18	1	2
IV	3	15	13	4
			1	4

Table 6
Recurrences and retreatment

Primary tumor	Recurrences		No re-treated	Average time between treatments (months)	Improvement rate
	No	Per cent			
Lung	17/81	14.8	6	10.8	5/6
Breast	6/14	42.8	3	5.3	3/3
Melanoma	1/6	16.6	—	—	—
Unknown	1/8	12.5	1	—	1/1
Total	20/113	17.7	10		9/10

Table 7
Comparison of the present results with other series

Series	No. of cases	Dose (Gy)	Improvement rate (per cent)	Median (months)	Mean (months)	One year (per cent)	Per cent retreated
CHAO et coll (1964)	38	30-40/3-5 weeks	63	—	8.2+ 4.6++	—	10.5
ORDER et coll (1968)	108	25-55/2-5½ weeks	60	—	6.3	16	14.8
NISCÉ et coll (1971)	376	35/3-4 weeks	80	~6	—	~16	17.5
JAZY & ARON (1974)	18	10 single dose	55	—	3.2	0	—
MONTANA et coll (1971)	47	30/2 weeks to 45/4½ weeks	56	~4	—	~12	—
SHIHATA et coll (1974)	81	10 single dose*	69	—	5.0	~7.5	47
HERRON et coll (1971)	44	40/3-4 weeks	61	3.5	—	—	—
PERNER (1974)	44	—	—	4	3.5	9	—
BERGHE et coll (1981)	910 (1st)	30-40/2-4 weeks	47	4.5	—	—	—
	907 (2nd)	20-40/1-3 weeks	52	3.8	—	—	—
Present series	113	30/1½ weeks	60	3	5.3	13	8.8

* 75 per cent of cases

67 per cent of cases

80 per cent of cases

+ responders: survival from onset of intracranial symptoms

non-responders: survival from onset of intracranial symptoms

the cases with metastases from lung tumors is not known although in 5 of the retreated cases the primary tumor was controlled. The initial response to retreatment was good in 9 of the 10 patients with recurrence although the duration of remission after retreatment was quite variable. Four cases with metastases from lung tumors and one patient with metastasis from a breast tumor are still alive 2 to 3½ months after retreatment. The other patients lived from 1 to 9 months after retreatment.

The overall survival rate in this series determined from start of treatment was 50 per cent at 3 months

and 13 per cent at one year (Table 5). The 6-month survival was 29 per cent. The inclusion of the patients who did not complete their treatments and those who received 40 Gy did not influence the median or one year survival. The survival was the same with the 53 cases whose primary lesions were diagnosed at the same time as the brain metastases. The effect of surgery on survival of those patients who were given combined treatment was variable. The representative numbers were too small to warrant a meaningful analysis. However, 2 patients with metastases from lung tumors and good neuro-

tion even the combined treatment are alive months and 2 years after treatment

The incidence of acute complications was insignificant. Three patients had initial nausea and vomit had increased headache and one had lethargy after the use of decadron. These symptoms were relieved by increasing the steroid dose. One patient developed intense scalp erythema which was considered to be potentiated by concomitant chemotherapy using adriamycin.

Discussion

The review revealed the same percentage of significant short term palliation as other authors have reported (Table 7). It appears therefore that 30 Gy in 3 fractions is an adequate dose for this purpose. The achievement of 30 per cent of the present patients to be functional before treatment did not affect the palliative effect since all but 3 of them remained in that functional level after treatment with significant amelioration of their symptoms. This observation was considered an improvement as suggested by ORDER et al. The median survival of 3 months was shorter than in other series (BORGELT et coll 1980 HORTON et coll 1971 MONTANA et coll NEWMAN & HANSEN 1974 NICE et coll ORDER et coll POSNER 1974). This may be due to the large number of cases with metastases from lung tumors whose median survival reflected that of the whole patient population. The 1 year survival of the present patients was similar to the approximately 12 per cent in the series of MONTANA et coll.

The use of shorter courses of 10 Gy single dose or 5 Gy in 2 fractions as the initial palliative treatment has been reported by some authors (HINDO et coll JAZY & ARON SHEHATA et coll YOUNG et coll) showing the rate of initial neurologic improvement to be comparable to that obtained with lower daily dose fractionated schedules (Table 7). However these shorter courses were associated with more severe acute complications and shorter survival (HINDO et coll JAZY & ARON YOUNG et coll). Nevertheless there may still be place for this regimen in certain cases with poor overall prognosis and rapid deterioration. YOUNG et coll advise against such treatment courses in patients with markedly increased intracranial pressure.

The results of two randomized investigations comparing 5 treatment schedules from 20 Gy in 2 fractions to 40 Gy in 4 weeks have recently been re-

ported by BORGELT et coll. Comparable results were obtained in all the treatment schedules using different parameters. The rate of neurologic improvement was 47 per cent in the first series and 52 per cent in the second. A more rapid improvement was observed in patients who received the shortest treatment schedules (30 Gy/2 weeks in the first series and 20 Gy/1 week in the second series). Patients with a controlled primary tumor and with the brain as the only site of metastasis had a more favorable prognosis. It was suggested that these patients may benefit from higher doses. The present review revealed that some of the patients in the same category had recurrence of the brain metastases even up to 15 months after the initial brain irradiation. ORDER et coll also suggested that a higher initial dose may be necessary in selected cases to prevent retreatment and prolong the duration of improvement.

The optimum higher dose still remains to be determined. ORDER et coll suggested an additional 10 to 15 Gy with reduced ports in patients given 25 to 40 Gy to the whole brain initially. MONTANA (1979) gave 30 Gy in 2 weeks and added 6 Gy with a reduced port in cases with solitary lesion. A current series comparing 50 Gy in 4 weeks and 30 Gy in 2 weeks for the patients with favorable prognosis has been published by BORGELT et coll.

In those series where reirradiation was mentioned this was done in up to 47 per cent of their cases (Table 7). The number of retreated cases in those series indicated that not all of the patients with recurrences were reirradiated. The retreatment dose was usually not discussed in the other series although in those who used the single dose of 10 Gy it would be assumed that they used the same dose. In the present series only half of the patients with recurrences were treated from 15 Gy in a week and half to 30 Gy in 3 weeks were given with good results particularly in those patients who received a retreatment dose of at least 25 Gy. The size of the retreatment fields depended on the extent of the recurrent lesion. In most cases the whole brain had to be reirradiated.

The effect of surgery on the results of treatment was not clearly evident as the number of patients in this group was relatively small. The same observation was made by ORDER et coll in their series. MONTANA et coll did not find any significant difference in survival between surgery plus irradiation and irradiation alone. POSNER showed that patients treated by surgery and irradiation had a longer sur-

vival than those given either rapid or slow irradiation alone. Despite the variable results, surgery may continue to be indicated in cases where the nature of the lesion is uncertain and biopsy confirmation is necessary (POSNER, WILSON 1977). The improvement in the accuracy of scintigraphy and computed tomography in distinguishing metastatic disease from other intracranial lesions may further diminish the role of surgery in this aspect.

In summary, it appears that 30 Gy in 2 weeks is an effective palliative dose in most cases of brain metastases. However, in selected cases with good performance status, primary tumor controlled and the brain is the only site of metastasis, higher doses should be considered. Shorter high dose courses also may be used depending on the severity of the patient's general condition. The indications for surgery have to be considered on an individual basis.

SUMMARY

Thirty patients with brain metastases from a variety of primary tumors received radiation therapy. Twenty-five per cent received 30 Gy in 2 weeks. Twenty per cent were operated upon. Significant improvement was obtained in 60 per cent of the patients. Indications, retreatment and the need for further doses in selected cases are discussed.

Received 1981, from Cornelius I. C. Turalba, Department of Radiation Oncology and Biophysics, Eastern Virginia Medical School, 600 Gresham Drive, Norfolk, Virginia 23507, USA.

REFERENCES

- ASH, UPMAR, E. Metastatic tumors of the brain and their localization. *Acta med scand* 154 (1956) 1.
- BAKER, A. Metastatic tumors of the nervous system. *Arch Path* 34 (1942) 495.
- BORCHERT, B., GILDER, R., KRAMER, S., BRADY, L., CHANG, C., DAVIS, L., PIREZ, C. and HENDRICKSON, F. The palliation of brain metastases. Final results of the first two studies by the Radiation Therapy Oncology Group. *Int J Radiat Oncol Biol Phys* 6 (1980) 1.
- CHAO, J., PHILLIPS, R. and NICKSON, J. Roentgen ray therapy of cerebral metastasis. *Cancer* 7 (1954) 682.
- CHASON, J., WALKER, F. and LANDERS, J. Metastatic carcinoma in the central nervous system and the dorsal root ganglia. A prospective autopsy study. *Cancer* 16 (1963) 781.
- DAVIS, O. CT in the diagnosis of supratentorial tumors. *Semin Roentgenol* 12 (1977) 97.
- DEELEY, T. J. and RICE, J. M. Radiotherapy in the management of cerebral secondaries from bronchial carcinoma. *Lancet* 1 (1968) 1209.
- ENZMANN, D., KRAMER, R., NORMAN, D. and POLLOCK, A. Malignant melanoma metastatic to the central nervous system. *Neurology* 127 (1978) 177.
- GILDER, R., LEVINE, N. JR., KOO, A. and McDONALD, D. Metastatic tumor presenting as intracerebral hemorrhage. *Radiology* 124 (1977) 109.
- HENDRICKSON, F. The optimum schedule for palliative radiotherapy for metastatic brain cancer. *Int J Radiat Oncol Biol Phys* 2 (1977) 165.
- HINDO, W., DETRANA, F. III, LEF, M. and HENDRICKSON, F. Large dose irradiation in treatment of cerebral metastasis. *Cancer* 26 (1970) 138.
- HORTON, J., BAXTER, D. H. and OLSON, K. The management of metastases to the brain by irradiation with corticosteroids. *Amer J Roentgenol* 111 (1971) 33.
- JAZY, F. and ARON, B. Single dose irradiation in treatment of cerebral metastases from bronchial carcinoma. *Cancer* 34 (1974) 254.
- LESSE, S. and NETSKY, M. Metastases of neoplasms to the central nervous system and meninges. *Arch Neurol Psychiatr (Chic)* 72 (1954) 133.
- MCGEE, E. Surgical treatment of cerebral metastases from lung cancer. The effect on quality and duration of survival. *J Neurosurg* 35 (1971) 416.
- MONTAGUE, E. Palliative radiotherapy in the management of metastatic disease excluding breast cancer. In: *Textbook of radiotherapy*, p. 794. Edited by T. Fletcher, Lea & Febiger, Philadelphia, 1973.
- MONTANA, G. Personal communication, 1979.
- , MEACHAM, W. and CALDWELL, W. Brain irradiation for metastatic disease of lung origin. *Cancer* 19 (1974) 1477.
- NEWMAN, S. and HANSEN, H. Frequency diagnosis in treatment of brain metastases in 747 consecutive patients with bronchogenic carcinoma. *Cancer* 33 (1974) 492.
- NISC, HILARIS, V. and CHU, F. A review of experience with irradiation of brain metastases. *Amer J Roentgenol* 111 (1971) 329.
- ORDER, S., HELLMAN, S., VON ESEN, C. and KILGER, M. Improvement in quality of survival following whole brain irradiation for brain metastases. *Radiology* 91 (1968) 149.
- POSNER, J. Diagnosis and treatment of metastases to the brain. *Clin Bull Memorial Sloan Kettering Cancer C* 4 (1974) 47.
- and SHAPIRO, W. Brain tumor. Current status of treatment and its complications. *Arch Neurol (Chic)* (1975) 781.
- RASKIND, R., WEISS, S., MANNING, J. and WERLICH, R. Survival after surgical excision of single metastatic tumors. *Amer J Roentgenol* 111 (1971) 33.
- RHOTO, A. L. JR., FICHINE, J. and TER POGOSIAN, M. Metastatic tumors. Localization by radioisotope scanning. *J Neurol* 16 (1966) 264.
- RICHARDS, P. and MCKISSOCK, W. Intracranial metastases. *Brit med J* 1 (1963) 15.
- SHIHATA, W., HENDRICKSON, F. and HINDO, W. Radiotherapy in the management of cerebral secondaries from bronchial carcinoma. *Lancet* 1 (1968) 1209.

- fractionation technique and re-treatment of cerebral metastases by irradiation. *Cancer* 34 (1974) 257
- STREIBER T P Metastatic tumors of the brain from a neurosurgical point of view. *J Neurosurg* 11 (1954) 84
- WILSON C Brain metastases: The basis for surgical selection. *Int J Radiat Oncol Biol Phys* 2 (1977) 169
- YOUNG D, POSNER J, CHU F and NISCE L Rapid course RT of cerebral metastases: Results and complications. *Cancer* 34 (1974) 1069

TRANSIENT INTESTINAL ISCHAEMIA INDUCED BY DEGRADABLE STARCH MICROSPHERES

Experiments in the cat

K. LOTE, M. FØLLING, B. ROSENGREN, K. SVANES and J. LEKVEN

The basic concept of radiation therapy is tumour eradication without unacceptable injury to normal tissue. This is possible when the tumour is more sensitive to radiation than the normal tissues, i.e. when a therapeutic ratio exceeding one exists. Such a ratio can be raised by increasing tumour sensitivity or by decreasing normal tissue sensitivity.

The search for tumour specific sensitizers has not up to now resulted in clinically applicable methods. As oxygen plays an active role in the formation of free cytotoxic radicals mediating the biologic effect of commonly available ionizing radiation for clinical use (photons and electrons), the oxygen tension in the tissues can modify the radiation effect (GRAY et coll 1953). The sensitivity of hypoxic cells in tumours can theoretically be raised by hyperbaric oxygen treatment at the time of irradiation and the radiation sensitivity of the normal tissues can be decreased by methods causing hypoxia.

The hyperbaric oxygen treatment principle has been clinically tried (CHURCHILL DAVIDSON et coll 1957) but the results have not been convincing and because of complications this method is no longer used. Other methods increasing the oxygen content in the tissues by means of peroxide infusion have also been abandoned.

Protection of normal tissues by induction of hypoxia has recently been described. PENN et coll (1975) demonstrated effective hypoxic protection to radiation of the gut induced by clamping the mesenteric arteries in dogs. STECKEL et coll (1969a, b)

and JOHNSON et coll (1968) demonstrated a similar protection of the gut and kidneys using vasoconstricting agents.

ARFORS et coll (1976) in pigs induced a transient hypoxia in the small intestine by the injection of degradable starch microsphere (DSM) into the superior mesenteric artery. FORSBERG (1978a) demonstrated a radiation protective effect on the hind foot and the same author (1978b) as well as FORSBERG & JUNG (1978) found a similar effect in the small intestine of the rat after hypoxia induced by degradable starch microspheres. FORSBERG et coll (1979) also demonstrated this protective effect against the development of late fibrosis in the gut wall. These experiments clearly show the possibilities of the hypoxic method for radiation protection already discussed by FORSBERG (1978a).

In clinical radiation therapy the intestine and kidney are examples of comparatively highly sensitive tissues. Radiation injury to these organs with subsequent intestinal ulcerations, necrosis and stenosis or renal functional disturbances and hypertension may make it impossible to deliver a sufficient dose to intra abdominal or retroperitoneal tumours.

The possible clinical use of hypoxia induced by injected degradable starch microspheres for protection of normal tissues unavoidably exposed to ionizing radiation awaits simple reliable methods to monitor the induced ischaemia. It is not possible to

use blood flow meters or oxygen tension meters or directly observe the organ colour at repeated treatment sessions in patients.

The present investigation was intended to assess in the cat (1) the value of a scintigraphic procedure to monitor the degree and duration of intestinal blood flow obstruction induced by the injection of $^{99}\text{Tc}^m$ labelled degradable starch microspheres into the superior mesenteric artery (2) the intestinal ischaemia induced by the starch sphere injection and (3) the intestinal and hepatic degradation of the starch microspheres.

Materials and Methods

Animal preparation. Seven adult male or female cats weighing 3.0 to 5.1 kg were used. The animals were fed on water only during 12 hours before an experiment. The cats were anaesthetized with sodium pentobarbital (40 mg/kg) and kept under light endotracheal anaesthesia after tracheostomy. A cannula (OD 1.02 mm) was inserted into the inferior vena cava through a femoral vein. A second cannula was placed in the left ventricle of the heart through the right carotid artery and a third was inserted into the abdominal aorta through the left femoral artery and secured with its tip just above the bifurcation. A midline laparotomy was performed and the main trunk of the superior mesenteric artery was isolated and prepared for measurement of the flow. After isolation and ligation of the distal end of the small iliocecal branch of the superior mesenteric artery a plastic cannula (OD 0.63 mm) was inserted and advanced 3 to 5 mm in the proximal direction and fixed in this position with the cannula tip pointing into the main trunk of the superior mesenteric artery for injection of degradable starch microspheres. Care was taken to secure that the tip of the cannula and the electromagnetic flow probe in the proximal part of the superior mesenteric artery were separated by at least 2 cm. The arterial blood pressure and heart rate were recorded by means of a Statham pressure transducer P23De connected to a Hewlett Packard 7758A Recorder. The animals were placed on a heating pad and the rectal temperature maintained at 37 to 38°C.

Degradable starch microspheres (DSM) with diameters of $37.6 \pm 5.9 \mu\text{m}$ suspended in 0.9% saline to a concentration of 13.2×10^6 spheres/ml (generously supplied by Pharmacia AB Uppsala, Sweden) were used to produce mesenteric arteriolar occlu-

sion. Before each experiment about 5×10^5 spheres were labelled with $^{99}\text{Tc}^m\text{O}_4$ (DJURSÄTER 1979) to specific activity ranging from 1.5 to 16.3×10^4 Bq/mixed with unlabelled spheres in a Whirlmixer. When the microspheres were sedimented by centrifugation less than 10 per cent of $^{99}\text{Tc}^m$ label remained in the supernatant. The labelled DSM were then resuspended in saline before use. Each animal received a dose of 27×10^6 DSM in a volume of 1 ml. The injection time ranged from 75 to 105 seconds.

Carbonated microspheres (CMS 3 M Company St Paul Minnesota USA) $14.8 \pm 1.3 \mu\text{m}$ diameter and labelled with ^{45}Sc , ^{51}Cr , ^{86}Sr or ^{111}In were used to measure blood flow per g of tissue. The microspheres were suspended in 10% dextran with addition of Tween 80 to prevent aggregation. The suspension was sonicated and mixed thoroughly in a Whirlmixer immediately before the injection. The suspensions contained approximately 1.2×10^6 CMS per ml and were injected into the left ventricle of the heart in a dose of 0.2 ml/kg body weight.

Tissue blood flow was determined by intracardiac injection of CMS and simultaneous withdrawal of reference blood sample from the aorta at a constant rate as described by HEYMANN et al. (1977). The net activity of the blood and tissue samples was determined and the blood flow was calculated from the following formula:

$$QT = QR \times \frac{\text{cpm T}}{\text{cpm R}}$$

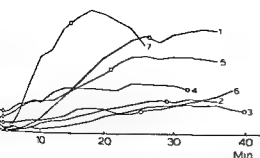
where QT = blood flow in tissue sample, QR = blood flow in aortic reference sample, cpm T = counts/min/g tissue, cpm R = counts/min in reference blood sample.

Cardiac output was also determined by the microsphere technique according to the following formula:

$$CO = QR \times \frac{\text{cpm I}}{\text{cpm R}}$$

where QR = blood flow in aortic cannula during withdrawal (1.03 ml/min), cpm I = counts/min of total amount of injected microspheres and cpm R = counts/min in reference blood sample. Tissue and blood samples were analysed in a multichannel gamma detector (ICN Instruments SC 772 Oakland, California USA).

Intestinal tissue samples were taken at 9 levels



Electromagnetically measured blood flow (ml/min) in the superior mesenteric artery before and following injection of $^{99}\text{Tc}^m$ labelled DSM into the artery. The symbol \circ denotes carbonized microsphere tissue flow determinations.

Table 1

Electromagnetically measured blood flow (ml/min) in the superior mesenteric artery following DSM injection

	DSM injection		
	Before	3 min after	26 min after
	105	2	140
	65	0	47.5
	110	3.5	38
	58	7.5	70
	87	28	112
	45	17	34
	87	5	175
SEM	79 \pm 9	10 \pm 4	81 \pm 17

0 to 90 cm from the ligamentum of Treitz. In sample the mucosa was separated from the muscularis by simple stripping and the two layers analysed separately. Microscopic examination showed that the mucosa samples were composed of the following layers: mucosa, muscularis, mucosae, submucosa. An occasional sample contained strips of smooth musculature. The muscularis samples contained only smooth musculature and the following.

amylase was determined by standard methods (ZINTERHOFER et al. 1973) and expressed in arbitrary units (normal range in man 40–140).

Imaging. Activity emitting from the abdominal area was recorded by a Pho/Gamma camera (Chicago, Illinois, USA) connected to a Nukab computer (Digital Equipment Corporation, Maynard, Massachusetts, USA). The intestinal and hepatic areas were identified by means

of a radioactive marker. By computer integration the accumulated number of counts over each area was determined at 3 min intervals for at least 27 min following the injection of $^{99}\text{Tc}^m$ labelled DSM into the superior mesenteric artery. During preliminary experiments it was confirmed that the CMS used for tissue flow determinations contributed by less than one per cent to total abdominal activity recorded by the gamma camera. The $^{99}\text{Tc}^m$ isotope in blood and tissue samples was permitted to decay for at least 7 days or 28 half lives and the samples were then counted for CMS activity.

Arterial blood flow. In the superior mesenteric artery was recorded by a flow probe with a diameter of 2.6 mm positioned over the artery and connected to a square wave electromagnetic flowmeter (Carolina Medical Electronics, 501 King, North Carolina, USA).

Experimental procedure. After instrumentation each animal was placed with the center of the abdomen upwards facing the center of the counting head of the gamma camera. The animal was not moved during the subsequent experiment. The first CMS injection was performed. The electromagnetic probe on the superior mesenteric artery recorded blood flow continuously. At time zero the $^{99}\text{Tc}^m$ labelled DSM were injected into the superior mesenteric artery through the cannula placed in its ileocaecal branch. The flow was recorded at one min intervals. From 2 to 4.5 min following the injection of DSM a second CMS tissue flow determination was carried out in each animal. After 14.5 to 40 min when the mesenteric flow was restored to a stable plateau as evaluated from the individual electromagnetic flow recordings over the superior mesenteric artery a third tissue flow determination was performed. The animal was then killed by intracardial injection of potassium chloride. The liver, pancreas, spleen and both kidneys were removed and immediately counted for total activity by the gamma camera.

Statistics. Each cat served as its own control. Student's two-tailed t test for paired data was used to calculate statistical probability. A common test for linear regression was also used for evaluation of the results. A p value less than 0.05 was considered statistically significant.

Results

Changes in arterial flow. The changes in arterial blood flow in the superior mesenteric artery in the

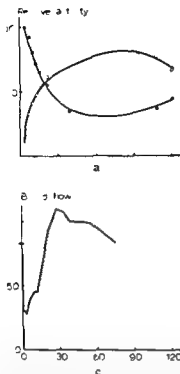
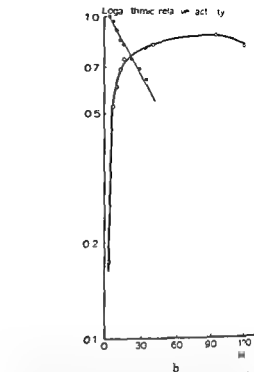


Fig. 2. Observations obtained in cat No. 5. a) Relative activity (in per cent) recorded over intestinal and hepatic areas 3 to 120 min following injection of Tc^{99m} labelled DSM into the superior mesenteric artery. Activity count over the intestinal area



0 to 3 min after DSM injection = 100% by definition. b) Semilogarithmic plot of the data presented in Fig. 4 (100% = 1 ml/min). c) Electromagnetically recorded blood flow (ml/min) in the superior mesenteric artery.

seven experimental animals appear in Fig. 1. Each animal was observed until the mesenteric blood flow reached pre-injection control levels or was stabilized. The mesenteric flow decreased to a minimum 1–11 to 4 min following DSM injection and gradually recovered thereafter. Control flow value was completely recovered 26 min after the DSM injection in 6 cats, with 3 animals having reactive hyperaemia. In one cat (No. 3, Table 1) complete recovery did not occur although flow increased markedly from the minimum level. One animal (No. 5) was observed for two hours and had a moderate reactive hyperaemia followed by a gradual decline towards control flow level, which was reached after 75 min (Fig. 2c). Although the variation between the individual cats was considerable, immediate and marked initial flow reduction after the DSM injection followed by flow restoration was consistently reproducible.

Changes in tissue flow. Intestinal CMS tissue flow was repeatedly determined at 9 consecutive levels in samples taken separately from the mucosa and the muscularis (Fig. 3). Immediately before DSM injection, a control CMS tissue flow determination was obtained 2 to 4.5 min after the DSM administration, the minimum flow level was determined. A recovery CMS tissue flow determination was performed when

the electromagnetic blood flow had reached either pre-DSM injection levels or a stable plateau phase. Due to individual variations in restoration of mesenteric arterial flow, the time for the third determination ranged from 14.5 to 40 min after the DSM injection (Fig. 1). The determination of the tissue flow demonstrated a highly significant reduction of the mucosal flow immediately following injection of DSM. In 2 of the cats the reduction in tissue flow in the distal ileum was much less than that observed in the proximal region.

A trend towards slightly reduced mucosal recovery flow in the jejunum and part of the ileum was evident when compared with pre-DSM injection control values. However, at no intestinal level did the difference reach statistical significance ($p > 0.05$). In contrast, tissue flow determinations in the muscularis regularly showed reactive hyperaemia, although the difference between control and recovery flow values only reached statistical significance at levels 10, 60 and 70 cm (normal to the ligamentum Treitz) (Fig. 3).

CMS tissue flow values in the liver, pancreas, spleen were not significantly altered by the DSM injection, and cardiac output remained unchanged (Table 2). However, renal tissue flow was significantly reduced. Thus, although intra-arterial injection

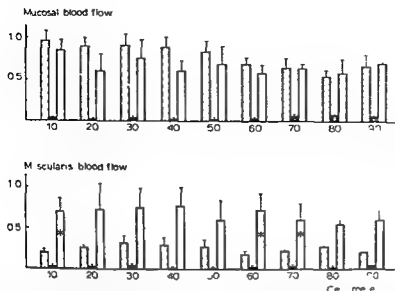


Fig. 3 Tissue flow (carbonized microsphere distribution) in small intestine mucosa (upper graph) and muscularis (lower graph) before (hatched) and 14.5 to 40 min (white) following injection of DSM into the superior mesenteric artery. Mean and SEM of 7 observations obtained at different distances aborally from the ligamentum of Treitz (in cm) are given. * statistically significant difference ($p < 0.05$) between control and recovery values.

Injection of DSM profoundly altered intestinal blood flow. The systemic circulation was not compromised except for a transient effect on the renal blood flow. The CMS tissue flow method and the electromagnetic method measure blood flow at separate levels in the arterial circulation. For each experimental animal three pairs of simultaneous CMS tissue flow determinations and arterial probe flow determinations were obtained demonstrating a positive correlation between the two methods (Fig. 4).

Degradation and distribution of starch microspheres. Emission from the hepatic and intestinal areas following $^{99}\text{Tc}^m$ labelled DSM injection was continuously recorded. Since the tracer must pass through the mesenteric vascular bed to reach the liver, intestinal activity during the time interval 0 to 3 min following DSM injection was defined as 100 per cent. Accordingly the relative activity remaining in the

intestinal area and the activity accumulating in the liver was calculated. It is recognized however that a minute fraction of $^{99}\text{Tc}^m$ indicator escaped from the intestinal to the hepatic area during the first 3 min interval making the defined 100 per cent slightly less than the amount of tracer actually injected into the superior mesenteric artery.

During the first 27 min following $^{99}\text{Tc}^m$ labelled DSM injection intestinal relative activity decreased while hepatic activity increased (Fig. 2a) in all animals. The individual semilogarithmic plots of intestinal relative activity transfer demonstrated initial exponential removal of $^{99}\text{Tc}^m$ and hence of DSM (Figs 2b, 5) while the liver showed rapid initial uptake of $^{99}\text{Tc}^m$ with a prolonged excretory phase (Fig. 2b). At the end of the experiment the liver had accumulated approximately 80 per cent of the total activity present in the liver, spleen, pancreas and

Table 2

Tissue flow (ml/min/g) and cardiac output (ml/min) (carbonized microsphere distribution) determined before (control) 2 to 4.5 min and 14.5 to 40 min after injection of $^{99}\text{Tc}^m$ labelled DSM into the superior mesenteric artery. Mean \pm SEM of 7 animals. Asterisk denotes a significant difference ($p < 0.05$) from control values.

DSM injection	Liver	Kidneys	Spleen	Pancreas	Cardiac output
Before	1.9 \pm 0.9	1.99 \pm 0.78	2.67 \pm 0.5	0.65 \pm 0.11	446 \pm 61
2 to 4.5 min after	1.13 \pm 0.48	1.0 \pm 0.1	2.66 \pm 0.48	0.56 \pm 0.15	478 \pm 55
14.5 to 40 min after	1.6 \pm 0.11	2.14 \pm 0.18	2.33 \pm 0.39	0.99 \pm 0.43	408 \pm 60

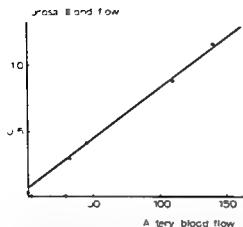


Fig. 4. Relationship between small intestine mucosal blood flow ($\text{ml}/\text{min}/\text{g}$) CMD and electromagnetically measured blood flow (ml/min) in the superior mesenteric artery. Determinations performed 10 times in each of 7 animals. Mucosal flow values represent mean of observations made at 9 levels of the small intestine.

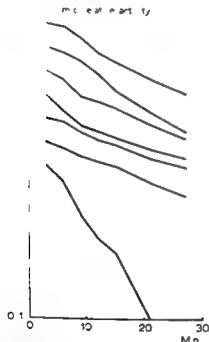


Fig. 5. Semilogarithmic plots. Decline of relative activity over the intestinal area following injection of ^{99}Tc labelled DSM into the superior mesenteric artery. Log activity counted over the intestinal area for 0 to 3 min after DSM injection = 1.0. The starting points for each curve on the ordinate scale are separated by a fixed interval for separation of the curves. Values on the ordinate scale are valid for cat No. 1.

kidneys (Table 3). The gamma camera images taken 3, 18 and 111 min following the injection of ^{99}Tc labelled DSM showed no abdominal hot spot outside the intestinal or hepatic areas (Fig. 7). One cat was observed for 120 min following the DSM injection and showed a late increase in intestinal activity with a concomitant decrease in hepatic activity indicating recirculation of tracer (Fig. 2c, b).

Table 3

Relative ^{99}Tc activity (per cent) in isolated abdominal organs counted by gamma camera after killing the animal. The sum of activity in the liver, pancreas, spleen and both kidneys defined as 100 per cent. Mean \pm SEM for 6 animals.

	Relative activity
Liver	80 ± 5
Pancreas	7 ± 1
Spleen	7 ± 0
Kidneys	11 ± 4

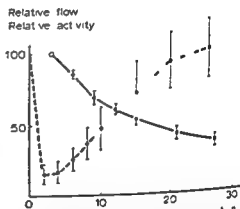


Fig. 6. Relative activity (per cent) recorded over an equal area (—) and relative blood flow (per cent) in the superior mesenteric artery (---) following injection of ^{99}Tc labelled DSM into the artery. Activity counted over intestinal area 0 to 3 min following and arterial flow preceding DSM injection is defined 100% respectively. Mean and SEM of 7 animals.

As relative activity recorded over the intestinal area decreased, relative arterial flow (per cent of pre DSM injection control value) in the superior mesenteric artery increased after a plateau of minimum flow lasting for 4 to 6 min (Fig. 6).

Serum amylase activity (mean 1450, range 1000–2100 arbitrary units) was not influenced by anaesthesia or DSM administration. Thus a dose-

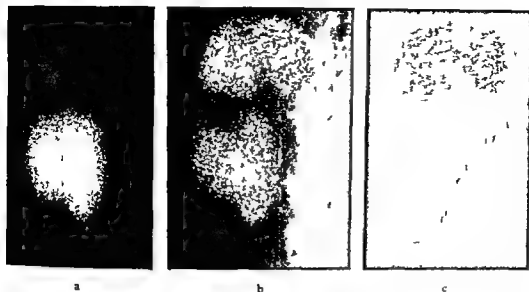


Fig. 7 Gamma camera images 3, 11 and 111 min following injection of ^{99m}Tc labelled DSM into the superior mesenteric artery.

terry demonstrating tracer transfer from the intestinal to the hepatic area (cat No. 5).

deceased cats possess 7 to 15 times higher amylase activity than do resting humans when the amylase activity is determined by the nephelometric method of ZINTERHOFER et coll.

Discussion

Temporary block of mesenteric blood flow in animals or man may be achieved by laparotomy and direct clamping of the superior mesenteric artery or by catheterization procedures using intra arterial inflatable balloons. The extensive collateral circulation made possible by the arcading mesenteric arteries is expected to shorten the length of small intestine rendered ischaemic by these methods. A theoretical advantage of degradable starch microspheres is that they will be carried by the blood stream through the arteries and finally embolize the small size arterioles (ARFORS et coll. FORSBERG 1978) by thus minimizing blood flow through collateral vessels.

In the present experiments selective DSM injection into the superior mesenteric artery immediately and markedly reduced blood flow in the main arterial trunk and in the intestinal mucosa and muscularis. In all but 2 animals blood flow was blocked along the small intestine from the duodenum to the caecum. When blood flow in the superior mesenteric artery is arrested mucosal oxygen is depleted in about 4 min in pigs (ARFORS et coll.) and extensive mucosal injury develops in 1 to 2 hours in animal

models (ROBINSON & MIRKOVITCH 1972 RIJKE et coll 1976) progressing to mesenterial infarction if ischaemia persists. However mesenteric arterial flow and blood flow in the intestinal mucosa and muscularis were restored at all levels of the small intestine after 26 min in the present experiments. Although one cat (No. 3) showed a 70 per cent reduction in arterial flow absolute recovery flow was sufficiently high to make intestinal ischaemic damage unlikely.

Intestinal muscle spasm induced by ischaemia (MARSTON 1963 ARFORS et coll.) would increase post ischaemic intestinal oxygen demand and could explain the reactive hyperaemia consistently found in the muscle layer.

Elimination of DSM from intestinal vessels depends on the separate processes of sphere degradation and wash out of polysaccharide fragments by the blood stream (ARFORS et coll. FORSBERG 1978b). The degradation rate is reflected in the exponential elimination of DSM linked ^{99m}Tc from the mesenteric vessels with an observed mean half time of 17 min. Individual results (e.g. cat No. 7) indicate that rapid elimination of DSM linked ^{99m}Tc from the intestinal vessels (Fig. 5) may be associated with early recovery of regional flow (Fig. 1 Table 1).

For any degree of DSM induced arteriolar block DSM linked tracer will always be retained in the mesenteric vessels until microsphere degradation permits passage through the capillaries. Blood flow can be measured by isotopic clearance methods if

the isotope is freely diffusible and does not itself influence regional flow (ZIERLER 1965). Neither condition applies to DSM linked $^{99}\text{Tc}^{\text{m}}$ clearance from the mesenteric vessels: thus exact calculation of intestinal blood flow from gamma camera counts is not possible. However, provided a total or near total arteriolar obstruction is induced, the rate of initial intestinal elimination may permit an estimate of the duration of the resulting intestinal ischaemia (Fig. 6). Early elimination of DSM linked $^{99}\text{Tc}^{\text{m}}$ was associated with recovery of intestinal blood flow.

After reaching the liver $^{99}\text{Tc}^{\text{m}}$ labelled mono- or poly-saccharides or starch fragments may be retained in hepatic glycogen stores or phagocytosed by Kupffer cells in the liver sinusoids, thus accounting for the observed rapid hepatic accumulation and excretion of activity (Fig. 2b). A continuous $^{99}\text{Tc}^{\text{m}}$ leakage into and redistribution by the systemic circulation would also be expected. The early marked deflection of hepatic activity from the straight line in the semilogarithmic plot (Fig. 2b) and the increase in hepatic activity illustrated in Fig. 6 confirm that such leakage did occur. The leakage of $^{99}\text{Tc}^{\text{m}}$ from the systemic circulation together with possible isotope excretion would also explain the observed simultaneous hepatic decrease and intestinal increase of activity after 120 min.

The present experiments differ from those previously reported concerning temporary regional ischaemia induced by DSM (ARFORS *et al.* 1978b, FORSBERG 1978b, FORSBERG *et al.* 1978a, FORSBERG & JUNG 1978, FORSBERG *et al.* 1978b, FORSBERG *et al.* 1979) in the following respects: a) the detailed tissue flow determinations performed separately in the intestinal mucosa and muscularis along the length of the small intestine; b) the experimental design using the gamma camera to obtain information about intestinal and hepatic degradation of $^{99}\text{Tc}^{\text{m}}$ labelled DSM; c) the choice of the cat as animal model. The results confirm and extend those of previous reports demonstrating that intra-arterial DSM injection rapidly and consistently induces a temporary circulatory block followed by full recovery of regional blood flow. While the regional use of vasoconstricting drugs to induce a similar degree of ischaemia may adversely affect the systemic circulation (FREEDMAN *et al.* 1978), the injections of DSM did not influence cardiac output.

The temporary minor decline in renal blood flow following DSM injection was probably caused by DSM spill-over from the superior mesenteric artery

into the aorta and subsequent DSM impaction in renal arterioles. Such spill-over into a blood vessel other than the injected one may confer hypoxic radiation protection to tumour bearing tissue and must be avoided.

A plateau of minimum arterial flow lasting for 6 min was achieved in all animals. Allowing a period of about 4 min to deplete mucosal oxygen (ARFORS *et al.*) approximately 2 min are left for delivery of abdominal irradiation while the small intestine holds hypoxic radiation protection. This is possible with modern linear accelerators delivering 4 min.

In man DSM dosage adjustments to the amylin activities met in human blood may be necessary to achieve a temporary flow obstruction of the desired duration. External detection of tracer activity injected with the DSM may offer the clinician a semi-quantitative non-invasive assessment of mesenteric blood flow and enable a selection of the appropriate time for radiation therapy.

SUMMARY

Temporary ischaemia was induced in cat small intestine by degradable starch microspheres. Regional arterial tissue blood flow immediately fell by 85 per cent, subsequent normalization within 26 min after microsphere injection. Degraded $^{99}\text{Tc}^{\text{m}}$ labelled microspheres were exponentially removed from intestinal vessels and accumulated in the liver. Provided near total arterial obstruction is induced, external isotope detection permits assessment of the duration of the induced ischaemia, which implies a selection of the appropriate time for radiation therapy.

ACKNOWLEDGEMENTS

Lill Andreassen, Arve Madsen and Inger Vikberg expert technical assistance. Determinations of serum amylase activities by the Department of Clinical Chemistry, Haukeland Sykehus are gratefully acknowledged as are grants from The Norwegian Cancer Society, The Norwegian Council for Science and the Humanistic Research Council, The Norwegian Council on Cardio-vascular Disease, Helga Semb's Legacy and Norsk Råd til Bakjempelen mot Fordøyelsessykdommer.

Request for reprints: Dr Knut Løte, Department of Radiology, Haukeland Sykehus, N-4016 Bergen, Norway.

REFERENCES

- ARFORS, K. E., FORSBERG, J. O., LARSEN, B., LEKVEN, J. H., ROSENGREN, B. and ÖDMAN, S. Temporary regional hypoxia induced by degradable microspheres. *Scand. J. Surg.* 262 (1976) 400.

1. KILL DAVIDSON I SANGER C and THOMLINSON R H Oxygenation in radiotherapy II Clinical application *Brit J Radiol* 30 (1957) 406
2. KESTER R Personal communication Pharmacia Lpda 1979
3. LEBERG J O (a) Transient ischemia for radioprotection. An experimental study with degradable microspheres *Acta Universitatis Upsaliensis* 1978
4. (b) Transient blood flow reduction by intra arterial injection of degradable starch microspheres Experiments in rats *Acta chir scand* 144 (1978) 275
5. (c) Transient blood flow reduction by intra aortal injection of starch microspheres Experiments in the rat *Acta radiol Oncol* 17 (1978) 353
6. JUNG H and JUNG B The protective effect of hypoxia against irradiation induced fibrosis in the rat *Acta radiol Oncology* 18 (1979) 65
7. JUNG B and LARSSON B (a) Modification of radiation response by degradable starch microspheres Experiments on the rat's foot *Acta radiol Oncology* 17 (1978) 359
8. (b) Mucosal protection during irradiation of extirpated rat ileum Effect of hypoxia induced by starch microspheres *Acta radiol Oncology* 17 (1978) 365
9. FREEDMAN A R KERR J C SWAN K G and HOBSON R W Primate mesenteric blood flow Effects of vasoconstriction and its route of delivery *Gastroenterology* 74 (1978) 875
10. GUY L H CONGER A D EBERT M HORNSEY S and SCOTT O C A The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy *Brit J Radiol* 26 (1953) 638
11. HEYMANN M A PAYNE B D HOFFMAN J I E and RUDOLPH A M Blood flow measurements with radioactive labeled particles *Progr Cardiovasc Dis* 20 (1977) 55
12. JOHNSON R E DOPPMAN J L HARBERT J C STECKEL R J and MACLOWRY J D Prevention of radiation nephritis with renal artery infusion of vasoconstrictors *Radiology* 91 (1968) 103
13. MARSTON A Causes of death in mesenteric arterial occlusion Local and general effects of devascularization of the bowel *Ann Surg* 158 (1963) 952
14. PENN T E PATTERSON V B and EDDY H A Protection of normal tissue during X irradiation by temporary hypoxia *Surg Forum* 6 (1975) 69
15. RIJKE R P C HANSEN W R PLAISIER H M and OSBORNE J W The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine *Gastroenterology* 71 (1976) 786
16. ROBINSON J W I and IRKOVITCH V The recovery of function and microcirculation in small intestinal loops following ischemia *G* 13 (1972) 784
17. STECKEL R J TOBIN P L STEIN J J and BENNETT R L (a) Intra arterial epinephrine protection against radiation nephritis *Radiology* 92 (1969) 1341
18. — ROSS G STEIN J J and STEVENS G (b) Radiation protection of vital organs using a selective arterial catheter *Amer J Roentgenol* 106 (1969) 841
19. ZIERLER K L Equations for measuring blood flow by external monitoring of radioisotopes *Circulat Res* 16 (1965) 309
20. ZINTERHOEFER L WARDLAW S JATLOW P and SELIGSON D Nephelometric determination of pancreatic enzymes I Amylase *Clin chim Acta* 43 (1973) 5

RADIATION THERAPY OF PRIMARY VAGINAL CARCINOMA

LUIGI PIRTOLI and RICCARDO SANTONI

Vaginal carcinoma is a rare tumor of elderly women. It accounts for about 1 to 2 per cent of all gynecologic malignant tumors with a mean age at onset of between 60 and 65 years (CHAU 1963, PARQUIER 1970, MURPHY 1971, UNDERWOOD & SMITH 1971, PEREZ et coll 1973, PREMPREE et coll 1977, GERBAULET et coll 1978).

Mainly due to its rarity there is a considerable lack of information concerning this neoplasm. Most reports describe a small number of cases collected over many years (DAW 1971, MARCUS et coll 1978). Moreover patients with carcinoma in situ and non epithelial tumor are sometimes grouped together with cases of primary invasive carcinoma as well as patients previously operated upon or irradiated for other gynecologic malignant tumors (BROWN et coll 1971, PEREZ et coll 1973, MARCUS et coll 1978).

Vaginal carcinoma was previously considered to have a poor prognosis (FRICK et coll 1968) but improved results have been reported in the past 10 years (BROWN et coll 1971, MURPHY, PREMPREE et coll 1977, MARCUS et coll 1978).

It has not yet been assessed whether surgery or radiation is to be preferred as the method of therapy. HERBST et coll (1970), DAW and UNDERWOOD & SMITH have reported series treated both with surgery and irradiation alone or in combination. However no definite conclusion can be drawn from these series as they are not comparable.

Surgery may be chosen in selected cases but of late causes severe complications (HERBST et coll 1970, UNDERWOOD & SMITH) due to the extended proce-

dures that is often required by the anatomic connections of the vagina (MARCUS et coll 1978).

Radiation therapy has been shown to be effective (BROWN et coll 1971, PREMPREE et coll 1977, MARCUS et coll 1978) but severe adverse effects may occur after irradiation with high doses (PEREZ et coll 1973, PREMPREE et coll 1977).

The results now reported indicate that carcinoma of the vagina is possible to control with radiation therapy without significant adverse effects.

Materials and Methods

The series consisted of 22 patients with primary vaginal carcinoma referred for radiation therapy to this institute from January 1965 to January 1975. The age ranged from 44 to 77 years with a maximum incidence between 70 and 75 years (8/22 i.e. 36.5%) mean age 65.

In all cases the microscopic diagnosis was invasive squamous cell carcinoma. The possibility that a vaginal localization of a primary arising elsewhere could have been diagnosed as a vaginal carcinoma was excluded for the following reasons: the uterine cervix was intact in all cases except in 2 patients in whom hysterectomy had been performed 6 and 10 years before referral for uterine leiomyofibroma (microscopically proven) and the vulva, urethra and anus were intact in all cases.

Besides vaginal and general examination at refer-

ral chest radiography urography barium enema cysto- and proctoscopy were performed in all cases.

Each patient was retrospectively assigned to a clinical stage according to the 1961 International Federation of Gynaecology and Obstetrics (FIGO) classification: Stage 0—Pre-invasive carcinoma (carcinoma in situ); Stage I—Tumor limited to the vaginal wall; Stage II—Tumor involving the sub-vaginal tissue but without extension to the pelvic wall; Stage III—Tumor extended to the pelvic wall; Stage IVa—Tumor involving the mucosa of the bladder or rectum with or without extension beyond the true pelvis; Stage IVb—Distant spread.

Sixteen patients had a tumor stage I, one a stage II, 4 a stage III and one a stage IVa. Inguinal metastatic nodes were present in 3 patients (2 with a stage I and one with a stage III tumor).

Surgical procedures were performed only for biopsy of the primary tumor and of the inguinal nodes.

A radical treatment was planned in 18 patients (13 stage I and 5 stage III). The treatment was only palliative in the remaining 4 patients (3 stage I and one stage III).

All patients undergoing radical treatment were irradiated with a cobalt or 31 MV roentgen rays from a source at a distance of 10 cm through anterior and posterior applicators including the pelvis and the vagina. Inguinal nodes were included only if metastatic or if the tumor extended to the lower third of the vagina. The minimum absorbed dose ranged from 50 to 60 Gy in 5 fractions (5 × 2 Gy per week).

A vaginal boost of a further 20 to 30 Gy to the mucosal surface with a radium loaded colpostat or a mould applicator for after loading technique (^{137}Cs or ^{192}Ir) was planned for each of the 13 patients with a tumor limited to the vagina and given radical irradiation. However, this type of irradiation was given in 8 cases only. It was not used in the remaining 7 patients due to a severe mucosal reaction of the vagina at the end of the external irradiation.

In the palliative treatments volume and beam arrangement varied according to the requirements of each clinical situation: the dose never exceeded 40 Gy in 4 weeks.

The condition of the patients was assessed at March 31, 1980. The minimum follow-up period was 5 years from the end of the irradiation: no patient was lost to follow-up.

The therapeutic response has been evaluated in terms of local control of the neoplastic mass and of

Table 1

Complete disappearance and 5 year survival correlated with the stage (of the tumor radically irradiated patients). Number of patients with metastatic inguinal nodes given in parentheses

Stage	Complete disappearance	5 year survival
I	11/13 (7%)	8/13 (17%)
II	0/1	0/1
III	2/4 (1/1)	2/4 (0/1)
Total	13/18	10/18
Per cent	72	55.5

Table 2

Complete disappearance and 5 year survival correlated with the type of treatment (radically irradiated patients stage I). Number of patients with metastatic inguinal nodes given in parentheses

Treatment	Complete disappearance	5 year survival
External irradiation only	6/7 (2/1)	5/7 (1/1)
External and endovaginal irradiation	5/6	3/6
Total	11/13	8/13

5 year survival. The adverse effects of the irradiation have been classified as slight, medium or severe.

Results and Discussion

The local control of the disease was evaluated within 2 months after the end of irradiation.

Complete disappearance of the tumor was achieved in 13/16 patients (i.e. 72%) given radical irradiation. In none of the 4 patients given palliative treatment was a complete objective response obtained: symptomatic relief was nevertheless attained in all four cases.

The overall 5 year survival amounts to about 55 per cent. In the radically irradiated patients a 5 year survival rate of 10/18 (i.e. 55.5%) was achieved. The patient given palliative irradiation survived for more than one year. All patients died because of the disease: having a local persistent or recurrent neoplastic mass.

Table 3

Five year survival rate after radiation therapy as reported in the past 10 years

Authors	Year	Stage distribution and survival				Overall survival
		I	II	III	IV	
BROWN et coll	1971	11/16	13/19	4/15	0/11	8/61 (46%)
GERBAULET et coll	1977	-	-	-	-	1/18 (30%)
PREMPREE et coll	1977	5/6	70/31	9/20	0/7	1/34 (5%)
MARCLUS et coll	1978	4/5	7/4	1/1	1/2	8/11 (67%)

The rate of complete disappearance and of 5 year survival correlated with the stage of the disease as shown in Table 1

Complete disappearance and 5 year survival is in correlation with the type of treatment (i.e. external irradiation alone versus external irradiation plus endovaginal irradiation) were analysed only for patients of stage I. The results appear in Table 2

Adverse effects Effects not lasting for more than 6 months and consisting of mucosal and cutaneous reactions, diarrhea and acute cystitis were classified as slight. They occurred in 15 patients and disappeared after medical treatment

Effects lasting for more than 3 months but controlled by medical treatment were classed as being of medium grade. They consisted of mucosal and cutaneous chronic abnormalities and hemorrhagic leukorrhea or cystitis and were observed in 3 patients

Severe adverse effects requiring surgical treatment such as stenosis, necrosis or fistulae did not occur in any patient

Radiation therapy of primary vaginal carcinoma has been reported to be successful both in terms of complete disappearance of the disease (91% MARCLUS et coll) and of survival (5 year survival rates as reported in the literature are listed in Table 3)

The present results with 72 per cent complete disappearance and 55.5 per cent 5 year survival for externally irradiated patients confirm that vaginal carcinoma can be cured by radiation therapy

The present series indicates that a good result can be achieved with radical irradiation particularly in the early stages of the disease (8/13 i.e. 61.5% 5 year survival rate in patients stage I) and that an additional vaginal boost dose does not seem to improve the results of external irradiation (3/6 versus 5/7 5 year survival rate)

Adverse effects of irradiation are reported to be minimal particularly for treatments with high

doses such as a combination of external endocavitary and interstitial irradiation with about 10 per cent severe complications (FRETZ et coll, PREMPREE et coll). Medium or slight adverse effects have not been specified in any of the reviewed reports

The adverse effects are low in the present series: no complication required surgery and only 3 cases had adverse effects of a medium grade

The present data indicate that radiation therapy can be adopted in many cases of primary carcinoma of the vagina with effectiveness and safety. This treatment is appropriate particularly for elderly patients who are in the majority with this type of tumor

SUMMARY

In a series of 22 patients with primary invasive squamous cell carcinoma of the vagina (stage I through IVa) a radical irradiation was planned in 18 and a palliative in the remaining 4 patients. The 5 year survival rate in the radically irradiated patients was 10/18 for all stages and 8/13 for patients of stage I. A vaginal boost irradiation did not seem to improve the results of external irradiation in patients of stage I. Severe adverse effects did not occur

REFERENCES

- BROWN G R, FLETCHER G H and RUTLEDGE F N. Irradiation of in situ and invasive squamous cell carcinomas of the vagina. *Cancer* 5 (1971) 1278
- CHAU P M. Radiotherapeutic management of malignant tumours of the vagina. *Amer J Roentgenol* 119 (1963) 502
- DAW M. Primary carcinoma of the vagina. *J Obstet Gynaec Brit Comm* 78 (1971) 853
- FRICK H C, JACOX H W and TAYLOR H C. Primary carcinoma of the vagina. *Amer J Obstet Gynec* 101 (1968) 695
- GERBAULET A, CHASSAGNE D, COSSET J M et COLLIN M C. Radiotherapie du cancer du vagin. Techniques et resultats a long terme. In *Le neoplasie dell apparato genitale femminile* p 51. Edited by U Veronesi. A

- Perussia F Di Re and Il Emanuelli Ambrosiana
Milano 1978
- HERBST A L GREEN JR T H and ULFELDER H Primary
carcinoma of the vagina An analysis of 68 cases
Amer J Obstet Gynec 106 (1970) 210
- MARCELS R II MILLION R R and DALY J W Carcino-
ma of the vagina Cancer 42 (1978) 2507
- MURPHY W T Primary carcinoma of the vagina *In*
Gynecological cancer p 214 Edited by T L Deeley
Butterworths London 1971
- PEREZ C A ARNISON A N GALANATOS A and
SAMANTH H K Malignant tumors of the vagina
Cancer 31 (1973) 36
- POURQUIER H Les epitheliomes primitifs du vagin
Radiol 13 (1970) 785
- PREMPREL T VIRAVATHAN A T SLAWSON R G W
BERG M J and CUCCIA C A Radiation manager
of primary carcinoma of the vagina Cancer 40 (1971)
109
- UNDERWOOD JR P B and SMITH R T Carcinoma of
vagina J Amer med Ass 217 (1971) 46

FROM THE DEPARTMENTS OF OTOTOLOGY AND RADIATION THERAPY UNIVERSITY HOSPITAL
DK 5000 ODENSE DENMARK

RELATIONSHIP BETWEEN HISTOLOGIC GRADING OF HEAD AND NECK TUMOURS AND REGRESSION AFTER CHEMOTHERAPY

A. JØRGENSEN and J. SCHLICHTING

In 1973 JAKOBSSON reported on the relationship between histologic grading and recurrence of squamous cell carcinoma after irradiation. Using a multistage histologic grading system he found a significant correlation in a series of 240 patients. LUND et coll (1976, 1977) modified the system and used it on series of carcinoma of the lip, tongue and larynx. They also found a significant relationship between histologic grading and the clinical result of treatment. However, the only practical consequence has been a suggestion of more frequent follow ups. It is likely that a certain histologic grade is correlated to the effect of certain schedules of treatment with cytostatic drugs. The present investigation was performed to elucidate this hypothesis.

Material. The series comprised 43 consecutive patients with 44 tumours of the head and neck (one patient had 2 tumours) all treated during the period from 1977 to Jan 1 1979 (Table 1). More than half of the cases had lymph node metastases in the neck at admission (Table 2). Two cases belonged to stage 1 (TNM-classification UICC 1978), 23 to stage 3 and 16 to stage 4. In 3 cases no staging was obtained and no case belonged to stage 1. The sex and age distribution appears in the Figure. All patients were given BIV-chemotherapy before irradiation. The tumour sizes were calculated from the surface of the primary tumour. Bleomycin 13 mg/m² intramuscularly, cyclophosphamide 70 mg/m² intravenously and Vincristine 1 mg/m² intravenously.

In order to obtain the best effect the drugs were administered according to the schedule given in Table 3. Before the beginning of the course and on day 12 the tumour shrinkage was evaluated and expressed in per cent, except in 3 cases where the evaluation was impossible because of the location of the tumours. Immediately after the chemotherapeutic course treatment with ⁶⁰Co was started. The aim was to administer 57 to 60 Gy during 6 weeks with 5 irradiations weekly using opposing fields, both irradiated each day. Details on the irradiation will be found in the report by JØRGENSEN & SELL (1971).

The histologic grading was performed on the primary biopsy material using the rules modified by LUND et coll (1977). The distribution of the cases according to the histologic score is given in Table 4.

Results

The results of the analysis appear in Table 5. The tumour shrinkage is expressed in per cent in 5 groups and the mean histologic score was calculated in each group. It is evident that no relationship existed between the tumour shrinkage and the histologic score. Independent of the method of analysis the result was the same.

Table 1

Location of primary tumour

Location	No of tumours
Oral cavity	6
Rhinopharynx	6
Oropharynx	17
Hypopharynx	4
Supraglottis	4
Glottis	9
Maxillary sinus	3
Total	44

Discussion

Previous publications concerning the relationship between histologic grading and clinical results of treatment have shown a significant correlation between histologic grading and recurrence of the disease (JAKOBSSON LUND *et coll* 1975a, b 1976 1977 WILLÉN *et coll* 1975). Possibly a relationship between histologic grading and tumour response after chemotherapy exists. Certain cytostatic drugs may influence tumours of a certain histologic grade. The drug used in the present series evidently had no effect on the tumours. A possible explanation is that the cytostatic drugs act in a complex way in the life cycle of the malignant cell (O'CONNOR *et coll* 1977). The treatment schedule (Table 3) was based on the fact that vincristine damages cells which are in the G₂ or premitotic phase resulting in an accumulation of mitosis 6 to 8 hours later. bleomycin is most injurious in the mitotic and G₂ phase and methotrexate has its greatest effect in the S phase. This complexity of the chemotherapeutic effects possibly does not permit any clear evaluation of the relationship between the histologic grading and tumour response. All previously published series except the one by HFLWEG LARSEN *et coll* (1978) suggest a correlation between the clinical results and histologic grading. Therefore in the future the histologic grading must be correlated to different principles of treatment. For the time being in this hospital it is planned to treat patients with epidermoid carcinomas with radiation sensitizers and correlate the results with the histologic grading.

Possibly a revision of the grading system itself by computer multifactorial analysis is motivated. The

Table 2

TNM classification (UICC 1978)

	No	N1	N2	N3
T1a	1	1	-	1
T1b	-	3	-	-
T2	1	1	1	1
T3	11	4	1	6
T4	3	3	-	1
Maxillary sinus	3	-	-	-
Total	19	13	2	10

Table 3

Schedule for treatment with cytostatic drugs (BLM = bleomycin, MTX = methotrexate, VCR = vincristine)

Day					
		1	5	8	1
8 a.m.	VCR			VCR	
1 p.m.	BLM+MTX	BLM		BLM+MTX	BLM

Table 4

Distribution of tumours in the different histologic grades

Score	No of tumours
1.50-1.99	7
2.00-2.49	9
2.50-2.99	14
3.00-3.49	14
3.50-4.00	5

Table 5

Relationship between tumour shrinkage after chemotherapy and histologic score

Shrinkage (per cent)	No of tumours	Mean histologic score
0-20	7	94
21-40	3	49
41-60	9	90
61-80	8	65
81-100	14	77
No evaluation	3	-

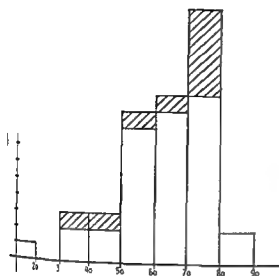


Fig. 1. Distribution at the time of histologic diagnosis. Hatched bars indicate females (n=9); white columns males (n=34).

inclusion of one or more less relevant parameters used and instead include other factors. WILLEN et coll actually reduced the system to 6 factors including the parameters structure and vascularisation. Using this modification WILLEN et coll found a clear correlation in a series of 124 patients with gingival carcinoma. Epidermoid carcinoma contains blood type iso antigens in quantities depending on their stage of differentiation (DABELSTEN et coll 1974); this fact may be a valuable supplement to the histologic grading. Other parameters such as the content of DNA (JAKOBSSON et coll 1975) might be useful and PAAVOLAINEN et coll (1973) have shown a relationship between the grade of malignancy and content of mucopolysaccharides of the stroma of epidermoid carcinoma.

Conclusion. Histologic grading has been reported to give a valuable supplementary information to the clinical evaluation of patients with head and neck tumours. However in the present series of 44 tumours no correlation was found between the effect of combined BMV treatment and histologic grading. Further testing of the grading system with other therapeutic regimes seems important and possibly attempts to revise the system.

SUMMARY

A series of 43 patients with 44 primary head and neck tumours were treated by combined BMV chemotherapy (bleomycin, methotrexate and vincristine) given before irradiation. The tumour shrinkages were evaluated and related to the histologic grading of the primary biopsy material. No relationship was found between the tumour response and degree of malignancy as evaluated by the histologic grading. The clinical relevance and possible modification of the histologic grading system are discussed.

REFERENCES

- DABELSTEN E, MØRÉN and HENRIKSEN B. Blood group substance A in carcinoma of the larynx. *Acta otolaryng* 77 (1974) 60.
- HELWEG LARSEN K, CARMELIEN, MLISTRUP LARSEN K, J and MFISTRUP L, KSEN U. Clinical relevance of histological grading of cancer of the larynx. *Acta path microbiol scand Sect A* 86 (1978) 499.
- JAKOBSSON P. Glottic carcinoma of the larynx. Thesis 1973.
- KILLANDER P, D. SILFVERSWARD C and WERSALL J. DNA contents of individual cells from squamous cell carcinomas of the larynx and tongue. *Laryngoscope* 85 (1975) 1701.
- JØRGENSEN K and SELL A. Carcinoma of the larynx. II. Treatment by ⁶⁰Co-irradiation. *Acta radiol Ther Phys Biol* 10 (1971) 161.
- LUND C, SØGAARD H, JØRGENSEN K and HJELMI HANSEN M. Epidermoid carcinoma of the larynx. VI. Histologic grading in the clinical evaluation. *Acta radiol Ther Phys Biol* 15 (1976) 293.
- ELBROND O, JØRGENSEN K and ANDERSEN A P. (a) Epidermoid carcinoma of the lip. Histologic grading in the clinical evaluation. *Acta radiol Ther Phys Biol* 14 (1975) 465.
- — — — — (b) Epidermoid carcinoma of the tongue. Histologic grading in the clinical evaluation. *Acta radiol Ther Phys Biol* 14 (1975) 513.
- JØRGENSEN K, ELBROND O, HJELMI HANSEN M and ANDERSEN A P. Histological grading of epidermoid carcinomas in the head and neck. *Dan med Bull* 24 (1977) 162.
- O'CONNOR A M, CLIFFORD P, DURDEN SMITH D, J, EDWARDS W, HOLLIS B A and DALLEY V M. Synchronous VBM and radiotherapy in the treatment of squamous cell carcinoma of the head and neck. *Clin Otolaryng* 1 (1977) 347.
- PAAVOLAINEN N, TARKKANEN J and SAKSFLA E. Stromal reactions as prognostic factors in epidermoid carcinoma of the tongue. *Acta otolaryng* 75 (1973) 316.
- WILLEN R, NATHANSON A, MOBERGER G and ANNEROTH G. Squamous cell carcinoma of the gingiva. *Acta otolaryng* 79 (1975) 146.

ABTEILUNG FÜR EXPERIMENTELLE TUMORFORSCHUNG (DIRECTOR J. SCHUMANN) FACHKLINIK
HORNHEIDE (DIRECTOR F. EHRLING) UNIVERSITÄT MÜNSTER D-4400 MÜNSTER GERMANY

EFFECTS OF ACUTE GAMMA IRRADIATION ON SPERMATOGENESIS AS REVEALED BY FLOW CYTOMETRY

U. HACKER, J. SCHUMANN and W. GÖHDE

A method rapidly revealing injury caused by irradiation or chemical compounds *in vivo* will be of great importance to ascertain cytotoxic and mutagenic effects. The cytofluorometric analysis provides such a method for measuring the amount of DNA in single suspended cells with a high degree of accuracy (MEISTRICH *et coll.* 1978a, GÖHDE *et coll.* 1979a).

The DNA distribution is related to the number of different cell types in a given tissue. In the testis the distribution reveals the number of cells in the different stages of spermatogenesis. Using cytometry of irradiated testes, alterations of the DNA-content of single cells should be possible to demonstrate changes in the cell kinetics of spermatogenesis, increase or decrease of the frequency of different cell types and mutations, alterations of the cell cycle and the cell phase progression of different cell types induced by different kinds of radiation have previously been demonstrated by pulse cytophotometry (GÖHDE 1973, GÖHDE 1976) but not the effects of gamma radiation on the phase progression, cell differentiation and induction of genetical aberrations of mammalian cells.

Material and Methods

Mice of the inbred strain NMRI aged 8 to 13 weeks were used.

Before the irradiation the mice were

anaesthetized by Evipan given intraperitoneally. The testes were then irradiated locally with 0.1, 0.25 or 0.5 Gy at a dose rate of 1.873 Gy/min or 1, 2.5, 5, 10 or 15 Gy at a dose rate of 4.125 Gy/min using a ^{60}Co -source. During the exposure the whole body with the exception of the testis region was shielded with lead. At doses below 1.0 Gy 4 experimental and 4 control mice were killed per dose and time point; at doses of 1.0 Gy and above at least 2 irradiated and 2 control mice were analysed.

Preparation of cell suspension. The testes were removed from fat and connective tissue. Both testes from each animal were minced using surgical blades and stirred 5 to 8 min in a pepsin solution (Pepsin Serva 130 Anson units/mg, pH 1.8) on a magnetic stirrer at room temperature (ZANTE *et coll.* 1976, ZANTE *et coll.* 1977). This preparation method results in a real single-cell suspension. The cells were neither washed nor centrifuged; thus selective cell loss was prevented.

Staining of DNA. The DNA of the cells was stained either with a dye solution containing 5 $\mu\text{g/ml}$ ethidium bromide (Serva Heidelberg), 12.5 $\mu\text{g/ml}$ mithramycin (Serva Heidelberg) and 1.5 mg/ml MgCl₂ in Tris buffer, pH 7.4 (ZANTE *et coll.* 1976, 1977) or with a DAPI dye solution (2.5 $\mu\text{g/ml}$ DAPI (4,6-Diamidino-2-phenylindol-2-HCl) in Tris buffer, pH 8.0, containing 0.5 mol MgCl₂ (GÖHDE *et coll.* 1979b). Both dyes result in identical fluorescence distributions (GÖHDE *et coll.* 1979b).

Flow cytometry: Measurements were performed with the new pulse cytophotometer developed by GÖHDE including the glass luminal flow chamber (GÖHDE et coll 1979 a)

DNA histograms: As reported in detail by ZANTE et coll (1977) the DNA fluorescence distributions of testicular cells obtained by the pulse cytophotometer are characterized by four peaks (Fig 3a). Peak III represents 2c (diploid) G_1 spermatogonia, secondary spermatocytes, Leydig cells and macrophages. This group normally comprises 12 to 17 per cent of a testis cell suspension. Peak IV is formed by cells with a 4c DNA content: primary spermatocytes, spermatogonia and probably some interstitial cells in the (G_2+M) phase. This peak normally contains 10 to 13 per cent of the testis cell suspension.

The S phase cells (spermatogonia: preleptotene spermatocytes, other S phase cells) between the 2c and 4c peak constitute 3 to 7 per cent of the testis cells. Two peaks with 1c (haploid) germ cells must be discriminated: round spermatids are represented in Peak II. They stoichiometrically with the 2c and 4c-cells and form about 40 per cent of the testis cells. Cells which are stored in Peak I show without a special enzymatic treatment about 70 per cent of the fluorescence intensity of cells registered in Peak II (ZANTE et coll 1977). Peak I represents elongated spermatids and spermatozoa (ZANTE et coll 1977) with the normal haploid DNA content. Their non proportional DNA staining is due to the highly condensed chromatin (MONLSI 1964, 1965). These cells normally comprise 24 to 32 per cent of a testicular cell suspension of an adult fertile mouse.

The 1c, S and 4c percentages were computed with the Interactive Spectrum Analyzing Program using a PAS Multichannel Analyzer and a PDP 11 computer (FRUH & GOLDING 1976). The percentages of the two groups of 1c cells were estimated by computing the area under each peak. Significance analysis was performed with the t test. Each analysed histogram consisted of about 50 000 measured cells.

Results

Irradiation heavily interferes with spermatogenesis as demonstrated by characteristic changes of the DNA distribution.

The alterations of the 2c, S, 4c and 1c-cell frequencies after acute gamma irradiation with 5 Gy appear in Fig. 1. At least two experimental and two

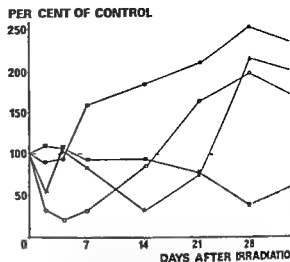


Fig. 1 Alterations of the percentages of the S (●), 4c (○) and the sum of the 1c-cells (■) of mouse testis at different intervals (7 to 35 days) after irradiation with 5 Gy in per cent of control.

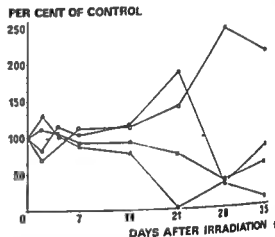
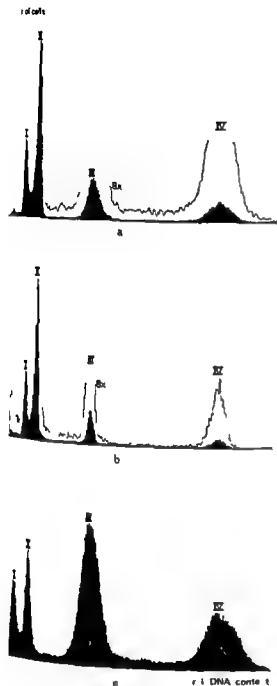


Fig. 2 Alterations of the percentages of round spermatids (●), elongated spermatids (■) and the sum of both cell types (○) of mouse testis at different intervals (7 to 35 days) after irradiation with 5 Gy in per cent of control.

control animals were killed on day 2, 4, 7, 14, 21 and 35 after irradiation. The average of the data from irradiated mice was expressed in per cent of the mean control figure. The total percentage of the haploid germ-cells (peak I and II of the DNA histograms) is given in the same figure. The most obvious effect shortly after irradiation was the immediate decrease of the number of S phase cells, about 1/5 of the control value during the first 7 days after the exposure (Fig. 1 a, b). At the same time the percentages of the 2c and 4c-cells were reduced to about 90 and 50 per cent respectively, the controls.



DNA distributions of mouse testis cells. Abscissa: Relative fluorescence values 756 channels. Ordinate: Number of cells per channel. a) Control mouse CV(II)=3.5%. Number of measured cells 30000. b) Mouse 2 days previously irradiated with 5 Gy CV(II)=3.5% CV(III)=2.2%. Number of measured cells 30000. c) Mouse 28 days previously irradiated with 5 Gy CV(II)=6.3% CV(III)=2.2%. Number of measured cells 150000.

the 2c-cells and the cells in proliferation (S phase) are reduced the relative number of the haploid cells increased. This indicates that the more differentiated spermatids (1c-cells) are less sensitive than the more primitive forms of spermatogenesis

(2c S 4c cells). After the irradiation the percentage of 2c cells increased and reached its highest point 28 days after the irradiation (2.5 times the control values). This relative increase was initially due to the decrease in S and 4c-cells and after the 14th day due to the reduction of haploid germ-cells.

The number of S-cells reached its lowest value (20% of control) after the irradiation. It remained constant until the 7th day after irradiation and then increased until it reached a maximum above the control value 28 days after the irradiation. The increase of the relative amount of S-phase reflects the recovery processes and is partially due to the decrease of 1c-cells at the same time. The percentage of 4c-cells increased above the control value 4 days after irradiation. It subsequently declined until it reached a minimum value (30% of control) 4 days after the exposure. This decrease of 4c-cells (mainly primary spermatocytes) is caused by the preceding killing of spermatogonia. Thereafter the number of 4c-cells increased again until it reached its highest point (about 200% of the control value) 28 days after the irradiation.

The alterations of the haploid germ-cells (cells of Peak I and II) that occur after irradiation with 5 Gy appear in Fig. 2. The total of the 2c S and 4c cells is also shown in the same figure. After 2 days and after 4 days the total percentage of haploid cells had increased significantly ($p < 0.05$) but none of the special haploid cell types was significantly altered during the first week after irradiation. From the 7th day after the exposure the percentage of sperm and elongated spermatids decreased below the control value and reached its lowest point 21 days after the irradiation. According to the time table of OAKBERG (1957) the elongated spermatids and sperm were spermatids 7 days earlier, spermatids and primary spermatocytes in late meiosis 14 days earlier and primary spermatocytes in early meiosis 21 days earlier. The round spermatids are reduced from the 7th day after exposure and disappear nearly completely after about 3 weeks. At the same time the 1c-cells are reduced to 75 per cent. This is a consequence of the sharp reduction of round spermatids irradiated as more mature spermatogonia which are extremely radiation sensitive (OAKBERG 1955b).

One week later the gap of missing cell types had shifted somewhat and involved elongated spermatids and sperm which were reduced to 1/3 of the control. At the same time the total percentage of

haploid germ cells reached the lowest value (35% of control) while the number of round spermatids increased again. In contrast to the reduction of the haploid germ-cells the percentage of 2c S and 4c cells had relatively increased above the control value.

The percentage of round spermatids along with that of all haploid germ-cells had 35 days after the irradiation increased while the number of elongated spermatids and sperm had further declined to 10 per cent of controls as a consequence of maturation depletion due to killed spermatogonia (Examples of the radiation induced alterations described appear in Fig. 3). The control histogram has the following distribution: elongated spermatids and sperm 27 per cent, round spermatids 38, 2c cells 17, S phase 6 and 4c cells 12 per cent. The coefficient of variation of Peak I is 5.5 per cent, of Peak II 3.5, of Peak III 4.7 and of Peak IV 3.5 per cent. The coefficient of variation (CV) of Peak I and Peak III are normally broader than that of Peak II, probably because Peak II is composed of only one subfraction (round spermatids) whereas the other two peaks represent cells with a different stainability of the nuclear material. Peak I contains elongated spermatids and sperm and Peak III is composed of 2c spermatogonia in different phases of maturation, secondary spermatocytes and some other cells (microphages, Sertoli cells, Leydig cells).

A histogram of the testes of a mouse 2 days after irradiation with 5 Gy is illustrated in Fig. 3b. The most obvious effects are the reduction of S phase (the upper line is multiplied by a factor of 8) to 1.2 per cent, the reduction of the 4c Peak to 6 per cent and the extreme narrow form of Peak III, which has a CV of only 2.2 while Peak II has a CV of 3 per cent. The reduction of the CV of Peak III was also observed after other radiation doses and may be a consequence of killing of one (or several) subfraction(s) of this peak, possibly the highly sensitive differentiated spermatogonia.

The situation 28 days after the irradiation is presented in Fig. 3c. Most obvious is the remarkable increase of Peak III (2c-cells) to 40 per cent, which was caused by a relative increase of interstitial and Sertoli cells due to the sharp reduction of haploid germ-cells (Peaks I and II). The increase of Peak IV (4c-cells) and in the S region was less marked than that of the 2c Peak. At this time the testes were atrophic and the weight was reduced to 20 per cent of the control value.

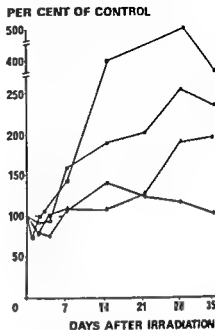


Fig. 4 Alterations of the percentage of 2c-cells after irradiation with 1 (●), 2.5 (▲), 5 (○) and 10 (■) Gy in per cent of control at different intervals (7 to 35 days) after the irradiation.

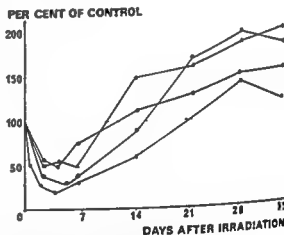


Fig. 5 Alterations of the percentage of S-phase cells after irradiation with 1 (●), 2.5 (▲), 5 (○) and 10 (■) Gy in per cent of control at different intervals (7 to 35 days) after the irradiation.

The number of 2c-cells after irradiation with different doses are demonstrated in Fig. 4. Following irradiation the 2c-cells initially and independently of the dose decreased due to killing of spermatogenic cells (spermatogonia). Several days later the number of 2c-cells increased in a dose-dependent manner with a maximum value of 5 times the control value 28 days after exposure. The increase of 2c-cells was accompanied by a dose-dependent reduction of the testis weight and is mostly due to the relative increase of interstitial and Sertoli cells.

PER CENT OF CONTROL

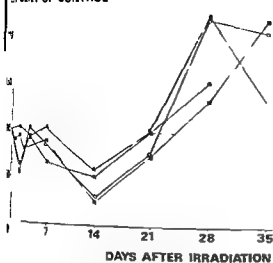


Fig. 6. Alterations of the percentage of 4c-cells after irradiation with 1 (●), 2.5 (▲), 5 (○) and 10 (■) Gy in per cent of control

PER CENT OF CONTROL

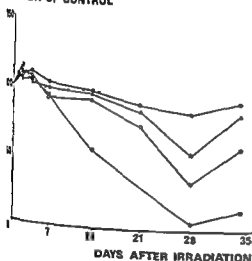


Fig. 7a

PER CENT OF CONTROL

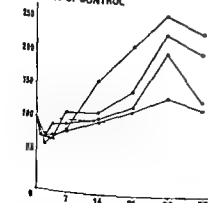


Fig. 7b

Fig. 7. Alterations of the percentage of a) haploid cells and b) 4c-cells after irradiation with 1 (●), 2.5 (▲), 5 (○) and 10 (■) Gy in per cent of control

(ITAGAKI 1970). This corresponds to the shrinkage of the seminiferous tubules as a consequence of the reduction of germ cells (Itagaki 1970).

The change of the S phase cell frequencies after irradiation appear in Fig. 5. The percentage of S phase decreased dose dependently during the first days. The recovery processes are dose-dependent but reduced at the highest dose.

The percentage of 4c-cells after irradiation is presented in Fig. 6. The 4c-cell frequencies appear to be nearly independent of the dose. After some fluctuations minimum values occurred after 14 days. These were due to killed mature spermatogonia which led to a sharp reduction of primary spermatocytes. Thereafter the percentage of 4c-cells increased and reached a highest value 28 days after irradiation with doses below 10 Gy and then declined again. At irradiation with 10 Gy the number of 4c-cells was still increasing 35 days later indicating that recovery processes after this high dose have not yet reached the same level as compared with the lower doses.

The total 1c-cell frequency of the testes after irradiation appears in Fig. 7a and those of the sum of the 2c + S and 4c-cells in Fig. 7b. Soon after the exposure all haploid cells increased relatively which was caused by the decrease of the 2c + S and 4c-cells. None of the effects had any strong dose dependency. Between the 4th and the 14th day until the 28th day the 1c-cell frequency decreased below the control value while the sum of the 2c + S and 4c-cells raised above the control value. Both effects were dose dependent. The first effect is a consequence of the reduction of spermatids irradiated as primary spermatocytes and spermatogonia. The second effect is initially caused by an increase of 2c-cells (relative increase of interstitial cells) and later by an increase of S and then 4c-cells (recovery processes and relative increase due to killing of germ-cells). Both effects are a consequence of the state of the testes: the more mature haploid germ cells which were in the stage of spermatogonia at the time of irradiation lead to a temporary depletion of the seminiferous epithelium with germ-cells. The 1c-cell proportion increased again 35 days after the irradiation and the total of the 2c + S and 4c-cells decreased. These dose-dependent effects indicate that the repopulation and the recovery processes already passed their maximum and that normalization was continuing.

A DNA histogram obtained 21 days after an ir

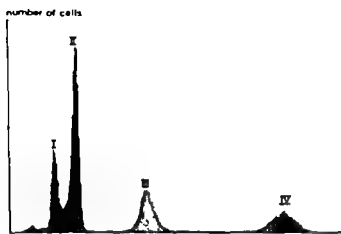


Fig 8a

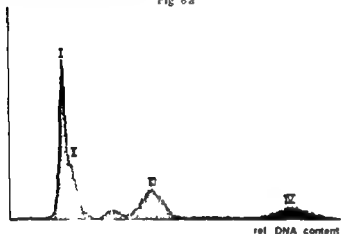


Fig 8b

radiation with 2.5 Gy is illustrated in Fig 8b. It is noticeable that the extra peak between the 1c and the 2c region represents. It shows two times the modal channel number of Peak I but is very broad (high coefficient of variation). If this peak would be caused by clumped cells, the broadness must be nearly the same as in the other peaks.

Fluorescence microscopy revealed that these cell suspensions contained abnormal oversized elongated spermatids and spermatozoa with two heads corresponding to the extra peak. Extra peaks were also observed in histograms of testis cells irradiated 14 days earlier. Cells that form elongated spermatids or sperm 14 or 21 days after irradiation were exposed as primary spermatocytes, which tend to form abnormal spermatids and sperm (OAKBERG & DI MINNO 1960). Round spermatids (Peak II) which were irradiated as mature spermatogonia had decreased to about 10 per cent (control more than 40%).

Another point of interest is the increase in the CV of the peaks in the histogram after irradiation indicating changes in the chromatin structure and possi-

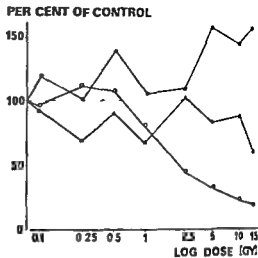


Fig 9

Fig 8 DNA-distributions of mouse testis cells: a) Control. Number of measured cells: 40,000. b) Mouse 21 days after irradiation with 2.5 Gy. Number of measured cells: 30,000.

Fig 9 Alterations of the percentages of the 2c (●) and 4c-cells (▲) 7 days after irradiation with different doses (0.1 to 15 Gy) in per cent of control.

bly also minor chromosome aberrations (Figs 3c).

The frequencies of the 2c, 3c and 4c-cells at 7th day after irradiation with different doses ranging from 0.1 to 15 Gy are demonstrated in Fig 9. At doses below 1 Gy, 4 experimental and 4 control mice were killed after doses of 1 Gy and above only experimental and 2 control mice were analysed each dose. The percentage of the 2c-cells was raised above the control value after all doses. Even at 0.1 Gy a significant ($p < 0.05$) relative increase of 2c cells occurred. This is a consequence of the diminution of S phase and 4c cells. The percentage of S phase cells was slightly reduced after 0.1 Gy and increased after 0.25 and 0.5 Gy. After higher doses the frequency of S phase cells diminished in a dose-related manner. This decrease does not show a simple exponential function. It is caused by several precursors of the cells now constituting S phase cells: more radiation resistant A_1 stem cells ($LD_{50} \approx 30$ Gy) and A_{2c} cells and the more radiation sensitive A_2 cells ($LD_{50} \approx 1$ Gy) and A_1 spermatogonia ($LD_{50} \approx 0.5$ Gy) (MEISTRICH et al. 1978a, OAKBERG 1971).

the percentage of the 4c-cells was reduced under control value after all doses except 2.5 Gy

Discussion

Several kinds of radiation effects that express themselves as alterations of the DNA distribution of testis cell suspension were detected

Decrease of 2c-cells shortly after irradiation was observed mainly by killing of differentiated 2c spermatogonia which are extremely sensitive in respect to radiation induced cell-death (OAKBERG 1955a, b)

At the same time a drastic decrease of S phase cells was observed caused by the killing of spermatogonia. This may be concluded from the fact that the minimum value of S phase was observed at the same time as a minimum value of spermatogonia (TWIG 1939, OAKBERG 1955a, b)

A decrease of early S phase cells after irradiation has also been observed by BRYAN & GOWEN (1956), MONESI & GUPTA & ILLIWA (1975)

A third effect appearing shortly after irradiation is the decrease of the 4c cell frequency which was a consequence of the cell killing of spermatogonia and of spermatocytes. No block in the G₂ phase as in other cell systems after irradiation was detected in testis cells

The reduction of these three cell types (2c, S, 4c) after irradiation led to a temporary relative increase of the haploid germ cells which had not yet reached late meiosis when they were more resistant to radiation than most other testicular cells (OAKBERG 1955a, b, OAKBERG & DI MINNO). The initial depletion of 2c, S- and 4c cells led to a dose dependent depletion of the seminiferous epithelium and of haploid germ cells. At first 2c spermatogonia died then the S phase cells, 4c cells rounded up and finally 28 days after the irradiation a number of elongated spermatids and spermatocytes diminished. (The S phase proportion is formed by spermatogonia and only to a small number of preleptotene spermatocytes whereas the S phase compartment is mainly composed of primary spermatocytes and only to a lower degree of spermatogonia). Thus the most obvious changes of the DNA-distribution as revealed by flow cytometric analysis were caused by cells in late spermatogonia. These cells normally die after irradiation in late interphase or mitotic phase (OAKBERG 1955a)

Besides proliferation a characteristic DNA distribution analysis also detects other radiation induced effects. Cells irradiated in the stage of primary spermatocytes do not divide immediately after the irradiation but tend to form abnormal spermatids (OAKBERG & DI MINNO). Obviously spermatids with irregular DNA content (with diploid DNA-cores) and chromosomal aberrations that are reflected by an increase of the CV can be detected by flow cytometric analysis

In addition the analysis of the CV of the peaks of the DNA histogram is a source of information. Thus the narrowing of the S phase peak two days after irradiation reflects killing of spermatogonia. The broadening of the peaks after irradiation showed that mutations occurred. These can be detected by flow cytometry only if the cells have divided

Regeneration processes appeared after all doses. At first the percentage of the S phase cells increased followed by an increase of 4c cells beginning approximately one week later. The percentage of haploid germ cells 35 days after the irradiation showed a dose-dependent increase following all doses

The disturbance of the testicular cytology after irradiation analysed with automated flow cytometric DNA estimation in single cells is in good agreement with the results of other much more time consuming methods (HERTWIG, OAKBERG 1955a, b)

The results show that the mouse spermatogenesis could be used as a biologic dosimeter: some of the cell stages have a relatively high sensitivity to radiation also in this flow cytometry test. Further investigations about the dose-dependent induction of changes of the maturation of spermatozoa, phase progression delay as well as mutagenesis (which is proportional to the increase of the CV of divided cells after irradiation) must be performed to establish the lowest dose detectable with the present technique. Nevertheless the results indicate that this dose may be less than 0.1 Gy

The analysis of radiation induced mutations can be a further application of flow cytometry. For this purpose it would be interesting to improve the resolution of the DNA measurements below 1 per cent of CV. This is only partially a technical problem because on homogeneous human and animal cell populations the accuracy of DNA measurements reaches CV values below 1 per cent (GÖRDE et al. 1979a). In the meantime the accu-

racy of the sperm DNA analysis with the pulse cytophotometer could be improved measurements with separated peaks for X and Y-chromosome bearing spermatids and sperm are now possible (MEISTRICH et coll 1978a OTTO et coll 1979)

SUMMARY

Mice irradiated with doses ranging from 0.1 to 15 Gy using a ⁶⁰Co source and controls were killed at intervals varying from 2 to 35 days after irradiation. The DNA content of the testicular cells in single cell suspensions was measured with the pulse cytophotometer to determine the frequencies of the different stages in the spermatogenesis. The relative amount of II phase and 4c cells was reduced initially but increased subsequently to hypernormal values. A decrease of 2c cells indicated a higher cell kill of diploid spermatogonia. Gamma ray induced spermatids with abnormal DNA values (diploid sperm) were identified. The results suggest that the spermatogenesis can be analysed with flow cytometry and used as a biologic dosimeter even for small doses of ionizing radiation.

ACKNOWLEDGEMENT

The author is indebted to Dr M. L. Meistrich, Houston, Texas, for critically revising the manuscript. This investigation is supported by a grant from the Arbeitskreis Krebsbekämpfung im Lande Nordrhein-Westfalen.

REFERENCES

- BARTOLIC G and GOWEN J W. A histological and morphometric study of the effects of X-rays on the mouse testis. *Biol. Bull.* 110 (1956) 229.
- FRUTKIN R and GARDINE F. Interactive spectrum analyzing program. In: Second international symposium on pulse-cytophotometry. Edited by W. Göhde, J. Schumann and Th. Buchner. European Press, Ghent, 1976.
- GÖHDE W. Zellzyklusanalysen mit dem Impuls cytophotometer. Der Einfluß chemischer und physikalischer Noxen auf die Proliferationskinetik von Tumorzellen. Habilitationsschrift, Münster, 1973.
- SCHUMANN J, BUCHNER TH, OTTO F J and BARLOCK H B. (a) Pulse-cytophotometry. Its application in tumor cell biology and clinical oncology. In: Flow cytometry and sorting. Edited by M. R. Melamed, P. F. Mullaney and M. L. Mendelsohn. John Wiley and Sons, New York, 1979.
- SCHUMANN J and ZANTI J. (b) The use of DAPI in pulse-cytophotometry. In: Third international symposium on pulse-cytophotometry. Edited by D. Lutz. European Press, Ghent, 1979.
- GUSTA G S and BAWA S R. Radiation effects on rat testes. VII. Alterations in nucleic acids and proteins. *Strahlentherapie* 149 (1975) 374.
- HERTWIG P. Die Regeneration des Samenepithels. Mäuse nach Röntgenbestrahlung unter besonderer Berücksichtigung der Spermatogonien. *Arch. Zellforsch.* 22 (1939) 68.
- ITAKAKI G. The effect of X-rays of 1000 R on germ cells in the mouse, with particular attention to the difference in X-ray sensitivity of spermatogonia. *J. Genetics* 45 (1970) 219.
- MEISTRICH M L, GÖHDE W, WHITE R A and SCHUMANN J. (a) Resolution of X and Y spermatid pulse cytophotometry. *Nature* 274 (1978) 871.
- HUNTER M R, SUZUKI N, TROSTI P K and WILKINS H R. (b) Gradual regeneration of mouse testis stem cells after exposure to ionizing radiation. *Radiat. Res.* 74 (1978) 349.
- MONTES V. Relation between X-ray sensitivity and stage of the cell cycle in spermatogonia in the mouse. *Radiat. Res.* 17 (1962) 809.
- Autoradiographic evidence of a nuclear histone synthesis during mouse spermatogenesis in the absence of detectable quantities of nuclear ribonucleic acid. *J. Cell Res.* 36 (1964) 683.
- Synthetic activities during spermatogenesis in mouse. RNA and protein. *Exp. Cell Res.* 49 (1978) 197.
- OAKBERG L F. (i) Degeneration of spermatogonia of a mouse following exposure to X-rays and stages in the mitotic cycle at which cell death occurs. *J. Morphol.* 93 (1955) 39.
- (b) Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of spermatogenesis in the mouse. *Radiat. Res.* 7 (1958) 367.
- Duration of spermatogenesis in the mouse. *Nature* 181 (1957) 1137.
- A new concept of spermatogonial stem cell renewal in the mouse and its relationship to genetic effects. *Mutat. Res.* 11 (1971) 1.
- and DI MINNO M. X-ray sensitivity of primary spermatocytes in the mouse. *Int. J. Rad. Biol.* 1 (1976) 196.
- OTTO F J and GÖHDE W. Effects of fast neutrons and X-ray irradiation on cell kinetics. In: Second international symposium on pulse cytophotometry. Edited by W. Göhde, J. Schumann and Th. Buchner. European Press, Ghent, 1976.
- HACKER U, ZANTI J, SCHUMANN J, GÖHDE W. Flow cytometry of human spermatozoa. *Humangenetik* 61 (1979) 249.
- ZANTI J, SCHUMANN J, BARLOCK H B, GÖHDE W and BUCHNER TH. New preparation and staining procedures for specific and rapid analysis of DNA in spermatozoa. In: Second international symposium on pulse cytophotometry. Edited by W. Göhde, J. Schumann and Th. Buchner. European Press, Ghent, 1976.
- SCHUMANN J, GÖHDE W and HACKER U. DNA fluorometry of mammalian sperm. *Histochemistry* 57 (1977) 1.

UPTAKE OF SEROTONIN LIBERATED BY RADIATION OF RABBITS AND MICE

TJEERD VENINGA and WILLY LEMSTRA

Since about 1960 considerable interest has been directed to radiation induced alterations in the content of endogenous compounds of several organs one of these being 5 hydroxytryptamine (5 HT) (RENSON & FISCHER 1959 MELCHING et al 1960 WILLOUGHBY 1960 RANDIĆ et coll 1962 ERSHOF & GAL 1961 ERSHOF et coll 1962 DEANOVIC & VENINGA 1962 DEANOVIC et coll 1963 MATSLOKA et coll 1966 SMITH & LANG 1966 PENTILLA & KORMANO 1971) This interest is free in the circulation in physiologically active and in some instances it may take part in biologic processes Normally it is for the greater part stored in a particulate fraction of cells like serotonergic enterochromaffin cells and blood platelets (CARATTINI & VALZELLI 1965) Previously it was reported that in rabbits and mice irradiation causes an immediate increase of plasma 5 HT originating from the intestinal tract (VENINGA et coll 1973) Since that time the interest in bio release processes after irradiation appeared to have declined probably because the reaction was thought to have no serious consequences Apart from that 5 HT has been suggested as a putative biochemical indicator of radiation injury (DEANOVIC 1971 STREFFER 1971 VENINGA 1971) However as it seems of interest to know whether 5 HT is of real significance for this reaction a series of experiments with different radiation doses was carried out in order to analyse the HT release in association with interfering pro-

Materials and Methods

Chinchilla rabbits and institute inbred grey mice have been irradiated under conditions as described previously (VENINGA et coll) Rabbits received an abdominal exposure whereas mice received either a total body or an abdominal irradiation In rabbits 3 ml aliquots of blood were collected from an ear vein 30 and 5 min before and 5 min after irradiation Murine blood was taken by orbital extraction immediately after the irradiation EDTA was used as anti coagulant Non irradiated animals served as controls The 5 HT level of plasma was determined by the method of ASHCROFT et coll (1964)

All animals were intraperitoneally injected 90 min before irradiation with 10 mg/kg iproniazide in order to prevent 5 HT break down A 10 cm piece of the small intestine 10 cm distal to the stomach was employed for determination of the amount of 5 HT in the intestinal lumen in mice The piece was excised immediately after irradiation and the lumen rinsed with 10 ml saline with the aid of a syringe The saline was collected in a Petri dish which after emptying was rinsed with another 10 ml saline 5 HT in the saline was determined according to the method described by BARCHAS et coll (1972) The mice were deprived of usual food for 48 h and drinking water for 36 h to omit contamination with the normal intestinal content Instead they received 24

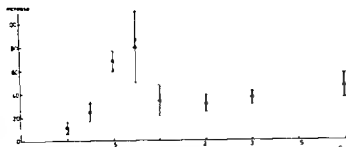


Fig. 1 Serotonin increments in the blood plasma of rabbits after different doses delivered to the abdomen. Values are expressed in percentages of the non irradiated controls (\pm SE).

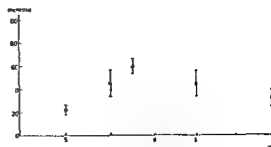


Fig. 2 Serotonin increments in blood plasma of mice after different doses delivered to the abdomen. Values are expressed in percentages of the non irradiated controls (\pm SE).

h before irradiation a solution containing 10 per cent $MgSO_4$ and 10 per cent sucrose. Intestinal pieces which nevertheless contained remainders of general food were discarded.

For the determination of 5 HT in murine spleen the organs were homogenized in ice cold saline (1:25) with an Ultraturrax (Janke und Kunkel Stufen W Germany). 5 HT was measured in the clear supernatant using the method of ASHCROFT et coll.

Results

In rabbit and mice irradiation of either the total body or the abdominal region gave rise to increased plasma 5 HT levels. In both animal species a linear dose response relationship existed over a range of roughly 0.75 Gy, starting in rabbits with a dose of 1 Gy, in mice with a dose of 5 Gy. Thereafter lower values for plasma 5 HT were found (Figs 1, 2). In a recent experiment with mice similar data were found except that the percentage 5 HT in the tissue was still higher, up to 90 per cent for 5.75 Gy thereafter lower values were observed again. In contrast to the other experiments this last experiment was carried out with female mice.

Doses below those indicated in the figures remained without effect. No 5 HT was detected in the intestinal lumen of mice after irradiation with doses up to 7.50 Gy. In contrast abdominal irradiation of mice resulted in a rise of 5 HT in the spleen (Table).

Discussion

The radiation induced release of 5 HT from the intestinal tract into the circulation was shown to be dose dependent but within a limited range. Rabbits and mice differed in their sensitivity for 5 HT release, the rabbits being much more sensitive. Radia-

Table
Content of 5 HT in spleen of mice under normal conditions as well as after exposure to different doses of radiation. The organs were excised immediately after exposure.

Dose (Gy)	No. of mice	5 HT (μ g/g spleen \pm SE)	Per cent rise (versus control)	p-value (versus control)
0 (control)	17	10.1 \pm 0.2		
5.5	17	1.8 \pm 0.6	77	<0.001
6.5	15	1.7 \pm 0.3	77	<0.001
7.5	19	1.3 \pm 0.5	31	<0.001

tion doses above those causing a linear response to lower plasma 5 HT levels as compared with data in the linear range. These lower levels can be explained on the basis of a second process which increasingly mobilized by higher doses, such as a diminution of 5 HT metabolism or 5 HT uptake, increased permeability of the intestinal wall due to radiation injury.

Augmentation of 5 HT metabolism is not unlikely since the metabolizing enzyme monoamine oxidase is inhibited by the presence of iproniazid. Stimulated uptake of 5 HT also seems improbable since it was shown that in vitro irradiation with doses up to 100 Gy caused no alterations in plasma values of 5 HT (VENINGA et coll.). Increased permeability of the intestinal wall to 5 HT immediately after the irradiation can be excluded since 5 HT was absent in the intestinal lumen before and after irradiation. These negative findings were not due to contamination with either $MgSO_4$ or sucrose since both substances had no influence on the determination of 5 HT in control experiments.

However, in mice remarkably higher 5 HT values

found in the spleen immediately after abdominal irradiation with doses ranging from 5.5 to 7.5 Gy (ARAGI *et al.* (1963) also reported higher 5 HT levels in the spleen of rats 24 h after 9 Gy γ irradiation. They also observed a nearly 20 per cent fall in red platelets at that time but this was not sufficient to explain the splenic rise in 5 HT. GERSHON & KRIS (1966) showed that accumulation of 5 HT in the spleen occurred in cells of the reticulo-endothelial system. The uptake of particles in this system was demonstrated to increase after irradiation (LEWING *et al.* 1970). Hence it might be possible to explain the present findings in the spleen by a reduced activity of the reticulo-endothelial cells in response to exposure to ionizing irradiation. The initially indicated suggestion of 5 HT as a biochemical indicator for radiation injury seems not to be of value in view of the irregular increase of 5 HT in blood plasma of both animal species.

SUMMARY

The radiation induced release of serotonin (5 HT) in rabbits and mice has a linear dose response relationship in a short range of approximately 0.75 Gy. In both animal species a threshold value is present which is 1 Gy for rabbits and 5 Gy for mice. Doses higher than those give rise to a linear range give rise to lower plasma 5 HT levels. These lower values occur concurrent with increased splenic 5 HT levels. Stimulated 5 HT uptake of the spleen may be responsible for the diminished increase in plasma 5 HT.

ACKNOWLEDGEMENT

The skilled technical assistance of Mrs H. Jansen is fully acknowledged.

REFERENCES

ARAGI G. W., CRAWFORD T. B. B., BINNS J. K. and McDONALD E. J. Estimation of 5 hydroxytryptamine in human blood. *Clin. chim. Acta* 9 (1964) 364.
 ARAGI G. W., ERDELYI E. and ANGIN P. Simultaneous determination of indole and catecholamines in tissues using a weak cation-exchange resin. *Analyt. Biochem.* 50 (1972) 1.
 KANAR R. and VENINGA T. S. Liberation of serotonin (and other amines) in the frog after X irradiation. *Int. J. Radiat. Biol.* 4 (1967) 249.
 LEWING Z. Les changements precoces du metabolisme des amines biogenes apres irradiation. In: *Biochemical indicators of radiation injury in man*, p. 113. IAEA panel proceedings series, Vienna 1971.
 S. PEK Z. and RANDIC M. Relationship between the dose of whole body X irradiation and the urinary excretion of 5 hydroxy indoleacetic acid in rats. *Int. J. Radiat. Biol.* 7 (1963) 1.

ERSHOF B. H. and GALE E. M. Effects of radiation on tissue serotonin level in rat. *Proc. Soc. exp. Biol.* 109 (1961) 160.
 — HELMERS R. and WEI J. F. Effect of a radio-protective agent on the uptake of serotonin in the X irradiated rat. *Proc. Soc. exp. Biol.* 116 (1962) 56.
 FLEMING K. E. and NODDERT W. The phagocytic activity of the reticuloendothelial system of mice following whole body X irradiation. *RES. J. Re. Nat. Acad. Sci. USA* 7 (1962) 1.
 GARATTINI S. and FELLER J. Serotonin. Elsevier, Amsterdam 1969.
 GERSHON M. D. and KRIS R. L. Location of sites of 5 hydroxytryptamine storage and metabolism by radioautograph. *Physiol.* 146 (1966) 477.
 HELMERS R. and WEI J. F. Effects of a radioprotective agent on the uptake of serotonin in the X irradiated rat. *Proc. Soc. exp. Biol.* 110 (1962) 536.
 MATSUKA O., TSUBOTA T., KASHIMA M. and ETO H. Serotonin and radiation effects on intestine. *Excerpta Medica Monographs on Nuclear Medicine and Biology*, No. 1, Amsterdam 1966.
 MELCHING H. J., ERDELYI E. and ROSLER H. Untersuchungen über einen biologischen Strahlenschutz. XXXVI. Zum Stoffwechsel des 5 Hydroxytryptamins bei der Ganzkörperbestrahlung weisser Mäuse und Ratten. *Strahlentherapie* 113 (1960) 394.
 PENTILLA A. and KORMANO M. Effect of X irradiation of the exteriorized jejunal loop on the morphology and 5 hydroxytryptamine content of the enterochromaffin cells in the mouse. *Strahlentherapie* 42 (1971) 238.
 RANDIC M., SUPRENANT Z. and LOVASEN Z. The influence of total body X irradiation on the 5 hydroxytryptamine content of the brain in normal rats. In: *Effects of ionizing radiation on the nervous system*, p. 263. IAEA, Vienna 1962.
 RENSON J. et FISCHER P. Liberation de 5 hydroxytryptamine par le rayonnement X. *Arch. int. Physiol.* 67 (1959) 142.
 SMITH H. and LANGLANDS A. O. Alterations in tryptophan metabolism in man after irradiation. *Int. J. Radiat. Biol.* 11 (1966) 487.
 STREFFER C. Biochemical post irradiation changes and radiation indicators. A review. IAEA panel proceedings series, p. 11. Vienna 1971.
 VARAGIĆ V., KRSTIĆ M., STEPANOVIĆ M. and HAJDUKOVIĆ S. The effect of γ irradiation on the amount of 5 hydroxytryptamine in the gut and spleen in the early phase after irradiation. *Experientia* 19 (1963) 647.
 VENINGA T. S. The significance of biogenic amines as radio indicators in experimental animals with reference to man. IAEA panel proceedings series, p. 175. Vienna 1971.
 — KERASTRA J. and WAGENAAR J. Origin of radiation released serotonin in rabbits and mice. *Acta radiol. Ther. Phys. Biol.* 12 (1973) 454.
 WILLOUGHBY D. A. Pharmacological aspects of the vascular permeability changes in the rat's intestine following abdominal radiation. *Brit. J. Radiol.* 33 (1960) 515.

THE SWEDISH RESEARCH INSTITUTE OF NATIONAL DEFENCE DEPARTMENT 1 S 10450 STOCKHOLM
 THE DEPARTMENT OF PATHOLOGY FACULTY OF VETERINARY MEDICINE SWEDISH UNIVERSITY OF
 AGRICULTURAL SCIENCES S 75007 UPPSALA SWEDEN

INDUCTION OF PITUITARY TUMOURS BY COMBINATION OF OESTROGENIC HORMONES AND ^{90}Sr

A NILSSON P BIERKE I HARALDSSON and A BROOME KARLSSON

Previously it has been shown (NILSSON & BIERKE 1973 NILSSON & BROOME KARLSSON) that oestrogenic hormones have a strongly inducing effect on the cancerogenicity of ^{90}Sr in mice. Whereas corticosteroid hormones have the opposite effect. Thus when oestrogen was given to mice treated with ^{90}Sr in a dose of 29.6 kBq (0.8 mg/kg body weight) in spite of a striking acute mortality the incidence of osteosarcomas increased twofold as compared with mice given ^{90}Sr alone (4.7 versus 2.3 tumours per mouse respectively). There was a concomitant deduction of the tumour induction time with about 40 per cent (194 ± 2.6 versus 311 days respectively). This striking effect of oestrogen did persist even if the ^{90}Sr dose was reduced to 14.8 kBq/g body weight to female mice as well as by a tumour frequency of 4.1 versus 2.0 and an induction time of 251.0 ± 4.1 versus 379.3 ± 9.7 days respectively. On the other hand if methylprednisolone was given for the whole surviving time (second week to mice treated with 14.8 kBq/g body weight) the tumour incidence was reduced to 0.16 tumours/mouse as compared with 1.82 given ^{90}Sr alone. The mean tumour induction time (589 days) as well as the mean survival time (15 ± 18.8) in the group given combined treatment was significantly prolonged as compared with the group given ^{90}Sr alone (450 ± 13 and 388 ± 17.8 days). The present investigation was initiated in order to analyse in more detail the promoting and modifying effects of oestrogens and corticosteroids on the cancerogenicity of ^{90}Sr . A primary aim was to find a ^{90}Sr dose low enough not to induce osteosarcomas but sufficiently high to pro-

duce bone tumours when combined with oestrogens. On the other hand regarding corticosteroid hormones the ideal objective was a ^{90}Sr dose which produced tumours at a level low enough to be completely inhibited by the combination of hormone and ^{90}Sr . However during the elaboration of this material it was found that ^{90}Sr at the low levels used and in combination with oestrogen hormones as a side effect did induce pituitary tumours in an unexpectedly high frequency. It was therefore decided to describe this material in a separate publication. Unfortunately the present material has not been taken care of in such a way that immunohistochemical and fluorescence microscopy or electron microscopy could be applied. Therefore the present report will mainly be concentrated upon an analysis of the frequency of pituitary tumours in the different groups used and their light microscopic characteristics.

Materials and Methods

Three series (A, B and C) of 75 ± 3 days old CBA male mice were each given 3 different doses of ^{90}Sr (NO_3) intraperitoneally. One of these series (A) was given ^{90}Sr only, one (B) in addition with polyoestradiol phosphate (Estradurin, Leo) subcutaneously at 3 consecutive times with intervals of one month. The remaining series (C) was in addition to ^{90}Sr also given methylprednisolone (Depomedrone, Upjohn) 1 mg subcutaneously every second week at 10 consecutive occasions. A fourth series (D) of 70 animals was given only Estradurin. The experimental condi-

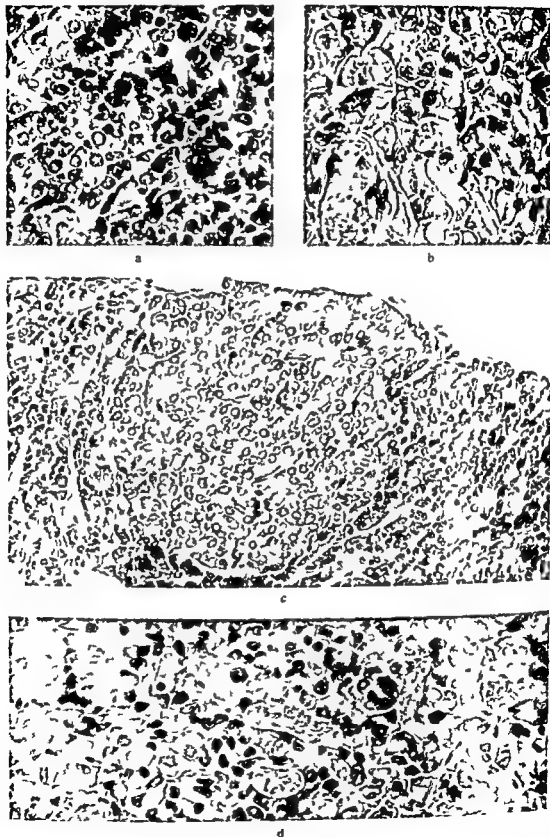


Fig. 1. a) Normal pituitary gland, pars distalis. Acidophilic cells are orange yellow, round or slightly ovoid with centrally placed nuclei. Basophilic cells are purple, slightly irregular with eccentrically placed nuclei. Tr-PAS $\times 480$. b) Pars distalis, 45 days after treatment with oestrogen + ^{90}Sr (7.4 kBq/g b.w.). Strong acidophilic hyperplasia. Slightly dilated sinusoids and

accumulation of basophilic collagen. Tr-PAS $\times 480$. c) Pars distalis, 540 days after treatment with oestrogen + ^{90}Sr (1.1 kBq/g b.w.). Nodular acidophilic hyperplasia. Tr-PAS $\times 480$. d) Pars distalis, 77 days after treatment with oestrogen + ^{90}Sr (1.1 kBq/g b.w.). Hyperaemia and sinusoidal dilatation. Presence of basophilic and chromophobic cells. Tr-PAS $\times 480$.

Table 1

procedures CBA male mice treated with $^{90}\text{Sr}(\text{NO}_3)_2$ oestrogenic (Estradiol) in the form of Depomedone hormone as given at different dose levels (subgroups 1, 2 and 3) and a 1 mmol/l intraperitoneal injection of ^{90}Sr (5 ± 3 days) (day 0). The hormones were given repeatedly by subcutaneous injection.

Subgroup	No of mice	^{90}Sr b.w. (kBq/ μCi) Day 0	Oestrogen (mg/mouse)			Corticosteroid (10 mg/mouse)							
			Day -7	7	51	Day -7	7	1	3	17	91	105	119
A1	100	0.975 (0.035)	-	-	-	-	-	-	-	-	-	-	-
A2	100	1.850 (0.050)	-	-	-	-	-	-	-	-	-	-	-
A3	50	7.400 (0.00)	-	-	-	-	-	-	-	-	-	-	-
B1	100	0.975 (0.035)	0.5	0.25	0.25	-	-	-	-	-	-	-	-
B2	100	1.850 (0.050)	0.5	0.25	0.25	-	-	-	-	-	-	-	-
B3	50	7.400 (0.000)	0.5	0.25	0.25	-	-	-	-	-	-	-	-
C1	100	0.975 (0.035)	-	-	-	+	+	-	-	-	+	+	+
C2	100	1.850 (0.050)	-	-	-	+	+	-	-	-	+	+	+
C3	50	7.400 (0.00)	-	-	-	+	+	-	-	-	+	+	+
D	-	-	1.0	0.5	0.25	-	-	-	-	-	-	-	-

the ^{90}Sr and hormone doses employed are given in Table 1. The mice (bred at the Swedish Institute of National Defence) were during the experimental period kept in the same room and inspected twice daily. They were grouped and maintained in cages with 10 animals each. A commercial pelleted diet (Standard feed) and mice (Astra Ewos) and water were available ad libitum.

When reaching a moribund state the mice were killed by cervical dislocation and examined by dorso-lateral radiography as a guide for locating the site of the hard tissue. The carcasses were then weighed as well as the liver, spleen, adrenals and kidneys. These organs, all macroscopically and soft tissue lesions, tumours and endologically suggested bone tumours were removed. The head was divided longitudinally close to the midline and fixed with the *in situ* Hard tissues including the skull were fixed in 70% formic acid. Ordinary histologic sections were used and the sections were stained with a mixture of eosin and with tri-PAS according to Pearce.

The skulls were treated as described by fixation in Steve's solution and decalcification procedure. This material was as previously depicted unsuitable for immunohistochemical studies. Therefore diagnosis of the pituitary tumours could not be based on their hormone secretory activity (LTH et coll. 1976) but had to be classified

according to their histologic features and affinity for stains. On these grounds they were divided into chromophobic, acidophilic and basophilic adenomas or in cases of malignancy as evidenced by infiltrative growth as carcinomas. The tumours were subdivided in three groups according to the histologic appearances: predominantly solid, sinusoidal or trabecular structure.

When comparing the tumour incidence between the different groups of mice the mean tumour induction rate (MTIR) was calculated. It was defined as the regression coefficient of the tumour frequency in 100 mice as a function of time between T-SD and T+SD, where T is the latency time between injection and the clinical appearance of the tumour and SD its standard deviation.

Results

Early abnormalities. An early histologic observation in mice treated with ^{90}Sr + oestrogenic hormone (series B) was as compared with a normal material (Fig. 1a) a more or less conspicuous dilatation of the pituitary sinusoids with a strong accumulation of blood (Figs 1d, 2). This initial congestion occurred in approximately the same frequency (15–25%) in all subgroups of series B but did appear somewhat earlier (40 days) in the group given the highest dose of ^{90}Sr (B 3) than in the two other groups (90–115 days).

Another general feature of this series (B) was an

acidophilic usually diffuse hyperplasia in numerous glands long before the first appearance of tumours (Fig 1b-c). In group B 3 this could be observed already about 50 days after the start of the experiment while in the other groups definite hyperplasia was evident first around day 250 to 275. A mere observation of the glands under low magnification easily revealed hyperplasia as a significant increase of the size of the pars anterior as compared with that of the normal gland cut in the same manner along the medial line (Figs 3-4).

Virtually in all cases the proliferating cells were acidophilic and generally somewhat larger than normally and were in the majority of glands diffusely distributed over the entire pars anterior (Fig 1b). In a few of these hyperplastic glands an additional finding was a strong accumulation of a basophilic homogeneous colloid in expanded sinusoids (Fig 1b-d). Considerably less frequent than the diffuse acidophilic hyperplasia was a strictly focal or nodular acidophilic hyperplasia which usually appeared at multiple sites within the glandular parenchyma (Fig 1c).

In the ^{90}Sr groups (series A) hyperplastic glands were not frequent and were in contrast to the II series generally chromophobic (6) or consisted of both chromophobic and acidophilic cells (3). Only one case with predominantly acidophilic cells was observed. In addition 2 cases of nodular hyperplasia were detected in the pars intermedia.

In series C hyperemia and sinusoidal dilatation was observed frequently. Hyperplasias of both chromophobic and acidophilic type were found at several occasions. In a few cases basophilic cells could also be found in an increased number as compared with normal glands. In series D acidophilic hyperplasia was fairly often observed as well as hyperemia and sinusoid dilatation.

Survival time The survival times of the mice in the different groups are presented in Table 2. Generally the mice in series B survived a much shorter time than in the mice of the corresponding dose groups of series A and C.

Tumour incidence The incidence of tumour bearing mice in the different groups is given in Table 2 as well as the tumour induction time and the mean tumour induction rate (MTIR). It was obvious from the figures that the combination of ^{90}Sr in low doses and oestrogenic hormones had a strong synergistic effect on the pituitary, a fact which highly significantly potentiated the effect of either treat-



Fig 2 Pituitary gland, pars distalis, 83 days after treatment with oestrogen + ^{90}Sr (0.925 kBq/g b.w.). Extreme sinusoidal dilatation and hyperemia. Trn PAS $\times 610$.

ment alone. A comparison of the whole ^{90}Sr male (A) with that of ^{90}Sr + oestrogens (B) and with oestrogen alone (D) revealed that the combination treatment was more effective than ^{90}Sr or oestrogen alone with almost a factor of 20 and 4 respectively. It was also obvious that corticosteroids (C) did not have much influence upon the induction rate of pituitary tumours (Table 2).

A comparison of the carcinogenic effect in the different groups of series B indicated an evaluation from the mean tumour induction rate in 100 mice (MTIR value) a clear difference between the groups (B 1 versus B 2, $p < 0.02$ and B 1 versus B 3, $p < 0.001$).

Tumour induction time The approximate latent time for tumour development (Table 2) was shown

Fig 3 Normal pituitary gland, 105 days old. Haematoxylin-eosin $\times 75$.

Fig 4 Pituitary gland, 4-6 days after treatment with oestrogen + ^{90}Sr (1.85 kBq/g b.w.). Hyperplasia, enlargement of pars distalis. Trn PAS $\times 75$.

Fig 5 Pituitary gland, 469 days after treatment with oestrogen + ^{90}Sr (1.85 kBq/g b.w.). Sinusoidal adenoma. Trn PAS $\times 610$.



Fig 3



Fig 4



Fig 5

Table 2

Incidence of pituitary tumours, mean induction time and mean tumour induction rate (MTIR) in CBA male mice treated with ^{90}Sr (group A), ^{90}Sr + oestrogenic hormone (group B), ^{90}Sr + glucocorticosteroid hormone (group C) and oestrogenic hormone (group D) as coded in Table 1

Series	Sub-group	No. of mice	Mean survival (days \pm SE)	Number of mice with tumour (%)	Mean induction time (days \pm SE)	MTIR (\pm SE)	t test B 1 and B 3 versus B 1
A	A 1	100	640 \pm 12	1 (1)	805		
	A 2	100	627 \pm 16	3 (3)	731		
	A 3	50	458 \pm 6	1 (2)	778		
B	B 1	100	446 \pm 25	44 (44)	641 \pm 14	0.166 \pm 0.009	
	B 2	100	500 \pm 18	37 (37)	629 \pm 15	0.144 \pm 0.009	p < 0.05
	B 3	50	380 \pm 30	15 (30)	543 \pm 23	0.178 \pm 0.010	p < 0.001
C	C 1	100	715 \pm 12	1 (1)	825		
	C 2	100	723 \pm 15	5 (5)	759		
	C 3	50	531 \pm 8	1 (2)	724		
D		70	502 \pm 21	7 (10)	670		

The mean tumour induction rate (MTIR) is defined as the regression coefficient of the tumour frequency in 100 mice as a function of time between T-SD and T+SD where T is the latency time between injection and the clinical appearance of the tumour and SD its standard deviation

in series B than in the other series. Within this series it was also evident that the tumours did appear significantly earlier with increasing dose of ^{90}Sr . Thus B 3 is clearly separated from both B 2 (p < 0.01) and B 1 (p < 0.001). However for B 1 and B 2 no difference was found.

Tumour types. The tumour material was classified according to its affinity to stains into predominantly acidophilic, basophilic or chromophobic types.

The tumours in series A and C all are chromophobic (Table 3) whereas in series B and D the tumours were either predominantly acidophilic or chromophobic. In series B it is also notable that the majority of the tumours (65.9%) were acidophilic in group B 1—like in series D (60.0%)—whereas the reversed situation was found in group B 2 (43.8%) and B 3 (13.3%). From the histologic appearance of the material in series B and D the impression was gained that these tumours were preceded by an acidophilic hyperplasia which progressively could be transformed into initially acidophilic adenomas which during their progressive growth successively turned out to become chromophobic. Therefore it was not always possible to classify the tumours as purely acidophilic or chromophobic since in most cases both types of cells were mixed. Thus the classification was based on the pre-

dominating cell type which means that a preponderance of chromophobic tumours could have acidophilic cell elements or vice versa. In many occasions the chromophobic cells gave an impression of being degranulated or very active, evaluated from their large golgi complex which sometimes contained a slightly pinkish or yellowish material. The histologic characteristics of the tumours could vary considerably within different areas but based on the predominance of a certain structure 3 different types were generally discernible. The most commonly observed type (Table 4, Fig. 5) was characterized by expanded sinusoidal structures surrounded by large ovoid or cylindrical cells with eccentrically placed nuclei (Fig. 6). The lumen of the sinusoids was filled with erythrocytes. Not infrequently the sinusoids were heavily dilated giving the tumours a cystic appearance particularly when several sinusoids were fused by rupture of their walls (haemorrhagic adenoma). In tumours of these types occasionally follicular like structures with indistinct or lacking lumen could be found. The vast majority of the tumours were of the sinusoidal cystic type (Table 4).

The second most frequent type was the predominantly solid tumour which mainly consisted of tightly packed small rounded or ovoid cells (Fig. 7).

Table 3

Incidence of pituitary tumours classified according to the predominant staining characteristics with tri PAS technique by Pearse. Treatment of the animal groups coded in Table 1

Series	Sub-group	No of mice	Tumours of pars distalis		Tumours of pars intermedia
			Acidophilic	Chromophobic	
A	A 1	100	—	1	
	A 2	100	—	3	
	A 3	50	—	1	—
B	B 1	100	29	15	—
	B 2	100	16	21	
	B 3	50	2	13	
C	C 1	100	—	1	—
	C 2	100	—	3	
	C 3	50	—	1	
D		70	5	2	—
Total		800	57	61	

The third type was in longitudinal sections made up of long slender strands (trabeculae) of tightly packed cells with a prominent intervening stroma against which the peripheral cells of the strands were arranged in a palisade like manner. On cross sections the tightly packed cells with indistinct cellular outline and rounded chromatin rich nuclei were forming rounded or irregular islands circumscribed by palisading cells along the stroma. In the centre of

these cell islands cells with somewhat excentrically placed nuclei could be found to form pseudofollicular structures consisting of a central blood vessel with palisading cells. These tumours were in the present context said to have a trabecular structure (Fig. 8).

On several occasions adenomatous tissues were found to contain small isolated clones of cells which contrasted with the majority of the cells by

Table 4

Incidence of pituitary tumours of the pars distalis classified according to the predominant histologic type. Treatment of the animal groups coded in Table 1

Series	Sub-group	No of mice	No of adenomas			No of carcinomas		
			Sinusoidal	Trabecular	Solid	Sinusoidal	Trabecular	Solid
A	A 1	100	—	—	1	—	—	—
	A 2	100	—	—	3	—	—	—
	A 3	50	—	—	1	—	—	—
B	B 1	100	31	1	5	4	—	3
	B 2	100	29	1	2	4	1	—
	B 3	50	8	1	1	3	—	2
C	C 1	100	—	—	—	—	—	1
	C 2	100	1	—	1	1	—	—
	C 3	50	1	—	—	—	—	—
D		70	4	—	—	3	—	—
Total		800	74	3	14	15	1	6



Fig 6 Pituitary acidophilic adenoma 537 days after treatment with oestrogen + ^{90}Sr (0.925 kBq/g b.w.) Sinusoidal structure predominantly acidophilic Tn PAS $\times 235$

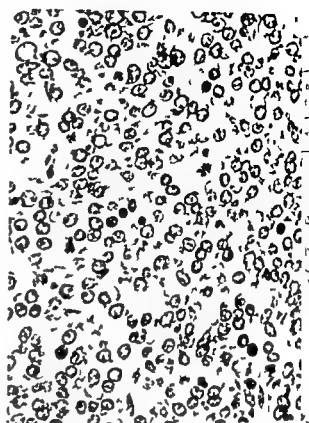


Fig 7 Pituitary chromophobic adenoma 617 days after treatment with oestrogen + ^{90}Sr (1.85 kBq/g b.w.) Solid structure Tn PAS $\times 605$



Fig 8 Pituitary chromophobic carcinoma 622 days after treatment with oestrogen + ^{90}Sr (1.85 kBq/g b.w.) Trabecular structure with cells palisading along vascular stroma Tn PAS $\times 200$

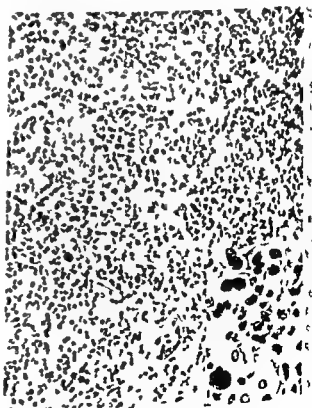


Fig 9 Acidophilic adenoma 417 days after treatment with oestrogen + ^{90}Sr (1.85 kBq/g b.w.) Central part: island of atypical predominantly chromophobic cells Tn-PAS 10 serial. Magnification of central part: nuclear hyperchromatism and mitotic figure $\times 510$

Table 5

mean number of pituitary tumours induced in groups of female CBA mice 75 \pm 5 days of age at the time of ^{90}Sr and oestrogen given

No. of mice	Dose of oestrogen and day of administration		Dose of ^{90}Sr (kBq/g b.w.)	Survival (mean \pm SE days)	No. of tumours	Remarks
	mg/mouse	Day				
50	—	—	14.8	388 \pm 17.8	1	
50	0.25	45, 75	—	369 \pm 30.9	1	
50	0.25	45, 75	14.8	253 \pm 9.3	1	
50	0.75	75, 105	14.8	89 \pm 7.8	0	
50	0.25	105, 135	14.8	377 \pm 17.3	1	Nilsson (unpublished evaluation)
50	0.25	135, 165	14.8	345 \pm 15.3	1	
50	0.25	255, 285	—	660 \pm 39.5	1	
50	0.75	755, 785	14.8	417 \pm 18.0	—	
50	—	—	—	873 \pm 77.7	—	
50	0.5	75, 105, 135	14.8	229 \pm 9.4	1	Nilsson & Broome & Karlsson (1976)
143	1.0, 0.5, 0.75	75, 105, 135	29.6	147 \pm 4.4	1	Nilsson & Ronnback (1973)

chromatin rich and atypical nuclei (Fig. 1). Of a total of 96 tumours in series B, 17 or 17.7 per cent were carcinomas as evidenced by pleomorphic atypical mitoses, capsular penetration (Figs 11) and infiltration of the surrounding tissues. A general impression was that the tumour cells during malignant transformation were losing their basophilic characteristics which seems to be mirrored by the fact that the majority (76.5% Table 4) of all carcinomas were chromophobic. The histology of the tumours could vary considerably from field to field as well as cytologic features. In some tumours multinucleated giant cells with large cytoplasmic inclusions of the nuclei were a frequent observation.

In series A and C all detected tumours were chromophobic. These tumours were generally in contrast to those induced by oestrogen or oestrogen and ^{90}Sr with their conspicuous sinusoidal dilatation and composed of very large polygonal or ovoid cells with a large cytoplasm and usually a prominent clear vacuole. The nuclei were prominently outlined and eccentrically placed and mitoses were frequently observed. In a few cases nuclear inclusions were discernible (Fig. 12).

Five tumours in group C 2.2 originated from the pars intermedia. One of these was a microscopic adenoma composed of small tightly packed basophilic cells with hyperchromatic nuclei and occasional mitoses (Fig. 13). The other was an adenoma

of macroscopic extent and consisted of slightly pleomorphic basophilic cells with a high frequency of nuclear inclusions.

Discussion

At an investigation originally performed for analysing the syncancerogenic effect of oestrogenic hormones and ^{90}Sr on bone tumour induction unexpectedly a very high incidence of pituitary tumours was observed. This was in contradiction to results obtained in several previous experiments in which ^{90}Sr was given in combination with oestrogenic hormones. However it is a wellknown fact that whole body irradiation, local irradiation of the skull or treatment with oestrogenic hormones alone or in combination with irradiation may induce pituitary tumours (UPTON & FURTH 1953; GARDNER et coll. 1953; CLIFTON 1959; KWA 1961; RUSSELL 1966; BERDUS 1967). Oestrogenic hormones readily induce pituitary tumours in rats and mice whereas rabbits, monkeys and guinea pigs are less susceptible (FURTH et coll. 1973). The majority of such tumours seem to originate from acidophilic cells (CLIFTON & MEYER 1956; LUNDIN & SCHELIN 1962) and they are generally considered to be benign (FURTH et coll. 1976). WAELBROECK, VAN GAVER & POTYLIK (1969) thus have found that pituitaries about 9 months after cessation of oestrogen treatment can recover indicating that the early adenomatoid



Fig 10



Fig 11



Fig 12



Fig 13

are oestrogen dependent. Tumours induced by external irradiation as well as by ^{90}Sr are usually epithelial (mammosomatotropic YOKORO et coll 1964). In addition they are generally more numerous than those induced by hormonal treatment (FURTH et coll 1976).

In reference to the brief survey given the present findings are not surprising but have none the less not previously been observed in experiments in which ^{90}Sr was combined with oestrogenic hormones. From the results presented in Table 2 it is evident that the frequency of pituitary tumours in the first groups of series B treated by oestrogen is closely related to the dose of ^{90}Sr i.e. the incidence of tumours is decreasing with increasing dose of ^{90}Sr . The reason for this is possibly associated with the fact that oestrogen renders the animals extremely sensitive to ^{90}Sr (NILSSON & RÖNNBACK 1971). Therefore with increasing doses of ^{90}Sr the survival time of the animals will be too short for tumours to develop in the pituitary. From the results presented in Tables 2 and 5 as regards tumour incidence and induction time there seems to be no doubt that ^{90}Sr strongly potentiates the effectiveness of oestrogen inducing tumours of the pituitary. It is provided that the ^{90}Sr dose is low enough to allow a survival time of sufficient length for tumours to develop. This also seems to be in agreement with the opinion (FURTH 1969) that the rationale of combination carcinogenesis is to bring about cell proliferation by some hormonal derangement and to hit the growing cells with mutagens which are most effective when the cells are in the DNA synthesis phase.

In order to evaluate the relationship between oestrogen and its influence upon the survival time and tumour frequency in oestrogen treated mice various experiments (Table 5) were analysed in

which higher doses have been present in the present series. From the results of the higher doses of ^{90}Sr it was concluded that oestrogen treated animals have a higher mortality and a reduced survival time and this will explain the results obtained. It has been found in

the present series that sinusoid dilatation was found in all series but was much earlier in the series treated by oestrogen alone. The reverse reaction may be considered as a reaction the degree of which may be related to the stress of the treatment. Another finding however occasional in this phase was the presence of a basophilic colloid material in the sinusoids. Both these findings agree with observations that the vascular supply of mammotropic tumours is abnormal (FURTH et coll 1976) and that many of the enlarged sinusoids characteristic of these tumours contain colloid material and exhibit vascular stasis (TIBOLDI et coll 1968). This reaction was in the oestrogen group followed by or partly replaced by a diffuse or sometimes multifocal acidophilic hyperplasia of varying degree. The hormonal secretion of these cells has not been tested. However the general opinion is that acidophilic cells are associated with mammotropic and somatotrophic function (SCHELIN et coll 1964). Furthermore most pituitary acidophilic tumours of the rat secrete both mammotropic and somatotrophic hormones and have therefore been named mammosomatotropic tumours (FURTH et coll 1976).

The observations in series B and D seem to indicate that a diffuse or nodular acidophilic hyperplasia may be a primary step in a successive development of acidophilic adenomas many of which will later lose their functional characteristics and turn chromophobic or transform into malignant tumours. This development is largely in agreement with the observation that a fluid transition between hyperplasia conditioned and autonomous neoplasia exists (FURTH et coll 1976).

In contrast to series B and D cells which functionally were of chromophobic type predominated clearly in series A and C.

Of a total of 115 tumours investigated only 2 were located in the pars intermedia whereas 52 acidophilic and 61 chromophobic were found in pars distalis. It is notable that all the tumours in series A (5) and C

§ 10 Pituitary acidophilic carcinoma. 516 days after treatment with oestrogen + ^{90}Sr (1.11 kBq/g b.w.). Infiltration and destruction of brain tissue and growth within the bone marrow. Very complex. Tn PAS x560.

§ 11 Pituitary chromophobic carcinoma. 614 days after treatment with oestrogen + ^{90}Sr (0.925 kBq/g b.w.). Strong nuclear atypia. Proliferation. Penetration through and bulging outside the capsule. Tn PAS x460.

§ 12 Pituitary chromophobic carcinoma. 778 days after treatment with oestrogen + ^{90}Sr (7.4 kBq/g b.w.). Chromophobic large cell tumour with glandular structure. Prominent golgi complex. Indistinct cell borders. Atypical mitotic figures. Tn PAS x400.

§ 13 Tumour of pars intermedia. 667 days after treatment with oestrogen + ^{90}Sr (4.1 kBq/g b.w.) and Depomedrone. Consisting of cells with distinct cell borders and small round or ovoid hyperchromatic nuclei. Atypical mitoses. Tn PAS x315.

(5) were chromophobic. In the B series the yield of acidophilic tumours was high (65.9%) in the group given the lowest dose of ^{90}Sr whereas in the intermediate and highest dose groups these figures were considerably reduced (43.2 and 13.4% respectively). The reason for this may be related to factors such as a decreased hormone production or degranulation of the transformed cells. The absence of acidophilia may also be related to a loss of information for specialized functions which is considered to take place during malignant transformation. The fact that not less than 13 of 17 carcinomas (76.4%) in the B series were chromophobic as well as the observation that malignant progression in acidophilic adenomas generally was evidenced by islands of chromophobic cells with dysplasia and atypical cell nuclei of varying degree may be considered as a theoretical support for this view. These observations are also in good agreement with previous findings that tumour cells in old acidophilic adenomas may lose their specific granules to such an extent that the acidophilic character may be overlooked (KRAUS 1953) and the fact that pituitary tumours which become malignant grow more or less chromophobic (FURTH et coll. 1976).

Tumours of pars intermedia are extremely rare in the small laboratory animals and seems to have been described only in rats (RUSSFIELD). On the other hand spontaneous tumours originating here are the most common types of pituitary tumours in horses and the second most common type in dogs (CAPEN 1978). In horses females are more frequently affected than males (GRIBBLE 1972). Most tumours in dogs are endocrinologically inactive although there may be cases with an excessive ACTH secretion. In horses a syndrome including muscular weakness, hyperidrosis, intermittent hyperpyrexia, hirsutism, polydipsia etc. develops mainly because the tumour exerts a pressure on the hypothalamic region (GRIBBLE). The two tumours detected in the present series were fairly small in size and have not demonstrated clinical symptoms.

The tumours of pars distalis were divided into 3 histologic types. The sinusoidal cystic tumours constituted an overwhelming majority which also seems to agree with the statement by WOLFF & WRIGHT (1947) that most of the smaller spontaneous adenomas are haemorrhagic and by FURTH et coll. (1976) who found the vascular supply of primary mammatropic adenomas to be abnormal. This also seems to be associated with the initial

hyperemia and stasis of the sinusoids which can be observed long before cellular proliferation and tumour transformation took place.

SUMMARY

The present investigation was initiated to analyze the carcinogenic effect of combined treatment with ^{90}Sr oestrogenic hormones or corticosteroids in inbred mice. Pituitary tumours appeared in a remarkably high incidence in mice treated with oestrogens + ^{90}Sr in doses—0.925 kBq/g body weight (44%) and 1.850 kBq/g body weight (37%)—as compared with mice treated with ^{90}Sr only—1 and 3 per cent respectively. The synergistic carcinogenic effect is ascribed to the oestrogen induced proliferation of pituitary cells and their increased sensitivity to radiation. The reverse relation found between number of pituitary tumours and dose of ^{90}Sr in oestrogen treated mice is explained by the reduction of the survival of the pituitary cells with increasing dose. Preneoplastic histologic changes in the pituitary are described and pituitary tumours which mainly appeared in pars distalis are classified according to their growth and functional characteristics.

ACKNOWLEDGEMENT

This investigation was supported by grants from the Swedish Cancer Society (Project No. 790-B 77 03 VC) and was also carried out as part of the program of the European Late Effects Project Group (EULEP).

REFERENCES

- BERDJIS C C: Pathogenesis of radiation induced endocrine tumors. *Oncology* 21 (1967) 49.
- CAPEN C C: Tumors of the endocrine glands. In *Tumors in domestic animals*, p. 377. Edited by J F Moore. University of California Press, Berkeley, Los Angeles, London 1978.
- CLIFTON K H: Problems in experimental tumorigenesis of the pituitary gland: gonads, adrenal cortex, mammary glands. A review. *Cancer Res.* 19 (1959) 1000.
- and MEYER R K: Mechanism of anterior pituitary tumour induction by oestrogen. *Anat. Res.* 125 (1965) 65.
- FURTH J: Pituitary cybrenetics and neoplasia. New Academic Press, Harvey Lect. 63 (1969) 47.
- NAKANE P and PASTILLIS J I: Tumours of the pituitary gland. In *Pathology of tumours of laboratory animals*, Volume 1. Tumours of the rat. Part 1. Edited by V S Turusov. International Agency for Research on Cancer, Lyon 1976.
- UEDA G and CLIFTON K: The pathophysiology of pituitaries and their tumours. Methodological advances. In *Methods in cancer research*, Volume 10. Edited by H Busch. Academic Press, New York 1973.

- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T In The physiopathology of
Second edition p 225 Edited by F Horn
and W H Fishman Harper (Hoeber) New
York 1963
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T In The endocrine system In Equine
medicine and surgery p 433 Edited by E J Catcott
R Smithcors Second American Veterinary Pub-
lishing Company 1972
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T In The pituitary gland (hypophysis) In
Endocrine system p 958 Edited by W A D
Graham and J H Rimpler London 1973
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T An experimental study of pituitary tumours
Springer Verlag Berlin New York 1961
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T and SCHELIN U Light and electron mi-
croscopic studies on the pituitary in stilbol treated
rats J Pathol microbiol scand 54 (1962) 66
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T and BROOME KARLSSON A Influence of
hormones on the carcinogenicity of ⁹⁰Sr Acta
Ther Phys Biol 15 (1976) 417
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T and BROOME KARLSSON A Influence of oestrogenic hormones
on the carcinogenicity and toxicity of radiostrontium Acta
Ther Phys Biol 12 (1973) 209
- W U PEEIFFER C A TRENTIN J J and
REYNOLDS J T Tumors of endocrine glands and second-
ary organs US Government printing office (Pub-
lishing Service Publications No 1332) Washington
1966
- SCHELIN U LUNDIN P M
and electron microscop-
stilbestrol induced pit-
crinology 75 (1964) 259
- TIBOLDI T KILZ M A F
blood supply of the
tumors in rats with
(1969) 259
- UPTON A C
by means of
84 (1953) 259
- WALBROECK A
hypophysaire
rats II Etu
(1969) 119
- WOLFF J M an
ous adenoma
Res 7 (1947) 9
- YOKORO K FURTH J and HUGHES N Induc-
tion of mammary pituitary tumors by X rays in rats
and mice The role of mammotropes Cancer Res 21
(1961) 178
- YOKORO K FURTH J and HUGHES N Tumor induction
with astrin 211 in rats Characterization of pituitary
tumors Cancer Res 24 (1964) 683
- YOKORO K FURTH J and HUGHES N Light
microscopic studies on the
autonomous
Endo
- YOKORO K FURTH J and HUGHES N Extraction of the
extracted pituitary
plasma 15
- YOKORO K FURTH J and HUGHES N Pituitary tumors
Exp Biol
- YOKORO K FURTH J and HUGHES N Tumeurs
endocrines chez les
europ J Cancer 5

INSTITUTE OF NEUROBIOLOGY UNIVERSITY OF GÖTHENBERG
 DEPARTMENT OF PHYSICAL BIOLOGY THE GUSTAF WERNER INSTITUTE
 GÖTEBORG SWEDEN AND THE DEPARTMENT OF THERAPEUTIC ONCOLOGY
 UNIVERSITY OF BERGEN N-5016 BERGEN NORWAY

EDEN
 ENALA
 YSCS

EFFECTS OF PROTON IRRADIATION OF THE LUMBAR SPINAL CORD ON INTRA AXONAL TRANSPORT OF ACETYLCHOLINE AND CHOLINERGIC ENZYMES IN RAT SCIATIC NERVE

S BOO, A DAHLSTRÖM, P A LARSSON, K ROSANDER and B P JENSEN

The sensitivity of peripheral nerves and ganglia to radiation has been analysed in many animal experiments. After irradiation with doses of 10 Gy and higher clear morphologic abnormalities have been observed in the peripheral nervous system (PNS) (NOTTER 1973). Functional and neurophysiologic changes related to morphologic lesions have also been observed (BERGSTRÖM 1962, CARSTEN & DAHLSTRÖM 1966).

Following postoperative irradiation of supraclavicular glands in patients with carcinoma of the breast functional disturbances as well as morphologic abnormalities in the nervous system may occur. Thus pareses and microscopically detectable changes were described after treatment with roentgen rays of 5 MeV (STOLL & ANDREWS 1966) and sensory and motor disturbances were observed in patients with breast carcinoma irradiated with ⁶⁰Co (NOTTER et coll 1972). Lesions of the plexus brachii were also noted in 17 per cent of such patients by NOTTER et coll (1970). The neurologic complications appeared in these patients if the dose was 50 Gy or more over a period of 30 days following the period between the irradiation and the appearance of the complications was between 5 months (STOLL & ANDREWS) and 20 months (NOTTER et coll).

Several factors are of importance but the larger

the total dose, the shorter the total irradiation time and the fewer fractions, the earlier do the symptoms and the morphologic lesions appear. The late neurologic symptoms may be due to direct or indirect irradiation effects. Direct irradiation trauma may for instance be due to a direct effect on the nervous tissue itself while indirect irradiation trauma may depend on disturbed nutrition of the nervous tissue caused by an impaired microcirculation.

Direct irradiation effects on the nervous tissue proper (i.e. the neurons) are difficult to demonstrate since the effects on blood vessels and surrounding glia always interfere with the neuron itself. One possibility is to observe the early effects of irradiation, i.e. before the time when circulatory changes occur. Glial reactions seem to occur within 24 hours after irradiation injury (cf. HAYMAKER 1969) which means that this effect is presumably impossible to separate from pure neuronal injuries.

One type of neuronal disturbance which might participate in the late neurologic symptoms is a changed intra axonal transport of various substances. In both adrenergic and cholinergic (motor and autonomic) neurons substances which are essential for the maintenance of nerve transmission and nerve terminal integrity are synthesized in the perikarya and transported by an efficient intra

axonal mechanism to the nerve terminals. Also a retrograde transport of material from the periphery of a neuron towards its soma exists. If the intraneuronal synthesis and transport of various substances (transmitter organelles, metabolizing enzymes etc.) were decreased because of direct irradiation effects on the soma, motor disturbances (or defects of the autonomic sympathetic or parasympathetic nervous system) would occur after a latency period. The intra-axonal transport of acetylcholine (ACh) and the cholinergic enzymes cholineacetyltransferase (CAT) and ACh-esterase (AChE) in a motor neuron system of rat has been investigated following local irradiation of the motor perikarya in the lumbar intumescence. The dose given was calculated in order to allow extrapolation of the results to clinical situations.

Materials and Methods

Ninety rats of the Sprague-Dawley strain (200–300 g, both sexes) were used. Under Nembutal anaesthesia and proper fixation, the lumbar intumescence of the animals was irradiated with a single dose of a 187 MeV proton beam from the 230 cm synchrocyclotron at the Gustaf Werner Institute in Uppsala (LARSSON *et al.* 1959). The field size was 3 mm × 8 mm and the absorbed single dose was 60 Gy. The dose rate was about 15 Gy/min. In one experiment in which the rats were used one day after irradiation 200 Gy were given. The field was adjusted before irradiation by a combination of conventional radiography and autoradiography and was controlled after irradiation by the same procedures (Fig. 1). Control animals were anaesthetized, fixed and radiography performed (short exposure) but not irradiated with the proton beam.

One, 7 or 30 days after the irradiation the animals were re-anaesthetized with ether and the sciatic nerves bilaterally were crush-operated at mid thigh level (the unbranched segment of the nerve) as described by LUBINSKA (1959). A silk ligature (3×0) was carefully introduced under the nerve and a glass rod with a diameter of 5 to 6 mm was placed on top of and along the nerve. The silk thread was then pulled firmly against the glass rod for 3 to 5 seconds. This procedure caused a clearly defined straight crush of the nerve, interrupting the axons but leaving the connective tissue sheaths intact. One or two such crushes (with 1 mm distance between



Fig. 1. The irradiated part of the spinal cord of the rat is denoted by the bright rectangle.

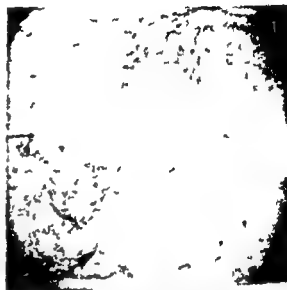
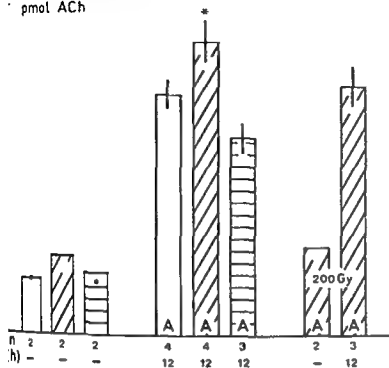


Fig. 2. Longitudinal section from the ventral part of the lumbar intumescence of a rat irradiated 30 days earlier having the severe paralysis of the rats in the group. Severe demyelination to the right and fairly normal appearance of the white matter to the left. Demarcation zone at arrow. (H&E-stain, 4×33).

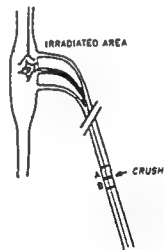
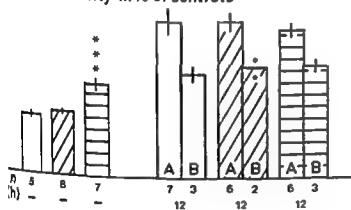
the 2 crushes) were made (Fig. 3). Some rats in the group were used unoperated, i.e. with intact sciatic nerves. In the experiments on day 30 (Fig. 4) nerves were crushed at 2 sites with 15 mm ap. Twelve hours after the nerve crush operation the rats were killed and 5 mm segments of the nerve relative to the crush site were removed. Before dividing the nerves in segments the sciatic nerves were carefully chilled on an ice-cooled glass plate.

Fig. 3. The levels of ACh and the activities of AChE and CAT in the sciatic nerve of control rats (white bars) and rats irradiated one day (bars with oblique stripes) or 7 days (bars with horizontal stripes) before the final experiment. In all cases 60 Gy was given except in one ACh experiment where 200 Gy was given (bars to the upper right). Individual observations (dots) means ± SEM (vertical bars) are indicated below the bars. Crush (h) which is indicated below the bars, crush (h). The local site of sciatic nerve crush and of the various nerve segments (1–4) of the sciatic nerve in relation to the irradiated area is shown in the illustration to the right.

pmol ACh



AChE activity in % of controls



CAT activity in % of controls

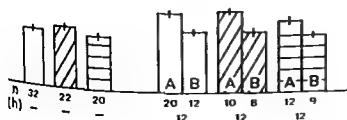


Fig 3

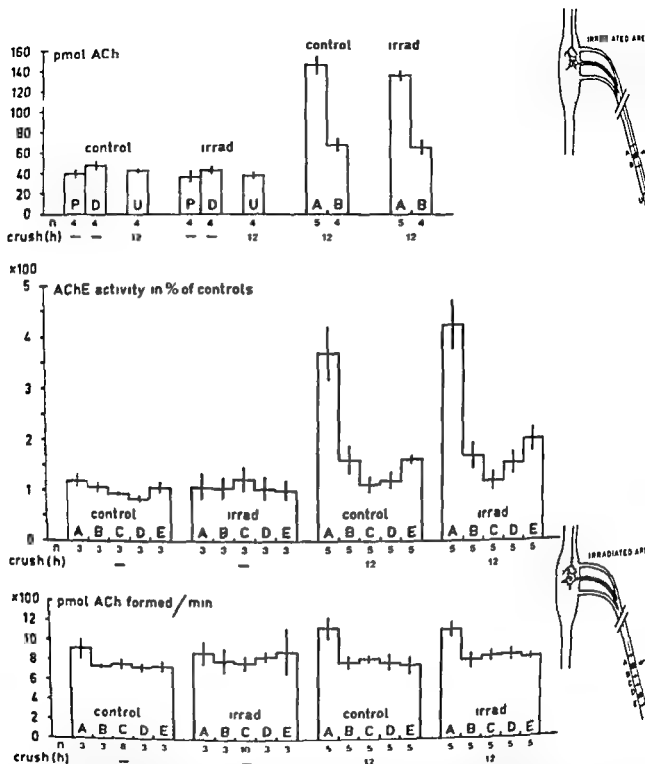


Fig. 4. The levels of ACh and activities of AChE and CAT in sciatic nerves of control rats and of rats irradiated 30 days before the final experiment. The values are given per 5 mm segment of nerve. Means \pm SEM (vertical bars) are indicated. Dose 60 Gy. n = number of experiments. Some nerves were crush-operated 12 h before killing the rats. crush (h). The location of the single

crush (ACh) or double crushes (enzymes) in relation to the various segments (A–T and U) are indicated in the small diagrams to the right. Letters P and D in the ACh bars for control nerves indicate proximal (P) part of nerve corresponding to A and B, and distal (D) part of nerve corresponding to D, F and U in crush-operated nerves.

Comparative segments were removed from the intact nerves (cf. Figs 3, 4).

For ACh assays 2 to 6 nerve segments were pooled. The ACh was extracted in TCA as described

by MACINTOSH & PERRY (1950) and used in the guinea pig ileum preparation (BLAIR & CLAYTON, 1961). An anti-histamine (mepyramine 2.5×10^{-5} M) was present in all solutions during the ACh

In the samples was identified by treatment of samples with purified AChE (Worthington ■ chemical New Jersey) and atropinization. Both procedures inhibited the response due to samples. The CAT activity was measured radiometrically according to GLOVER GREEN (1977) experiment on day 1 and 7 (Fig. 3) according to TUČEK (1974) in the experiment on day 30 (Fig. 4). AChE activity in experiments on day 1 and 7 (Fig. 3) was assayed as described by TAMM (1969) and in the experiments on day 30 according to TUČEK. With the latter method the activity of both enzymes could be measured in the same nerve segment homogenate.

The results were analysed using Student's *t* test. The lumbar intumescence of some control rats and of those in the 30-day group were removed and formalin embedded in paraffin, sectioned at 10 μ and stained with a routine stain. The sections were observed in a light microscope and photographed with Kodak TRI X Pan film (Fig. 2).

Results

No indication of irradiation injury could be seen in rats on day 1 or 7 but on day 30 one of 12 animals showed a marked paresis of the hind limbs and 5 a mild ataxic paresis. The remaining 6 of the 12 animals showed no evident symptoms. The spinal cord of these animals was examined in a light microscope. In the white substance a varying degree of demyelination was found in the irradiated zone, the most severe symptoms having a marked demyelination (Fig. 2). However the motor area of the anterior horn appeared to be normal in number and appearance. The rats with mild symptoms had a moderate degree of demyelination. Neurophysiology was normal in the control rats.

The ACh content of unoperated nerves appeared to be increased one day after irradiation with 60 Gy. When the irradiated nerves were compared with all control nerves (Fig. 3) the increase was significant ($p < 0.005$). The accumulated levels of ACh proximal to the crushes after 12 days were significantly higher than control in the rats given 60 Gy one day before the crush operation. On day 7 after irradiation the ACh content in both crushed nerves and nerves crushed 12 hours earlier was in the normal range (Fig. 3). In the experiment on day 30 no significant changes in ACh levels were observed when compared with the control

group. In the U segment the ACh content was 10 per cent lower than in the corresponding distal segment of uncrushed nerves in both the control group and the irradiated group (Fig. 4) indicating that the transportable fraction of ACh was about 10 per cent in both groups (cf. DAHLSTRÖM et al. 1974). No difference in ACh content or accumulation could be detected in the irradiated rats with no mild or severe neurologic symptoms.

The AChE activity in uncrushed nerve was 4600 ± 200 pmol of ACh split/min/5 mm ($100 \pm 6\%$) in the control group (Fig. 3) but increased to about 150 per cent on day 7 after irradiation ($p < 0.005$). No changes were observed in the enzyme accumulations in nerves crushed 12 hours earlier. In the experiment on day 30 the AChE activity of control uncrushed nerves was 4300 ± 600 pmol of ACh split/min/5 mm nerve. No clear gradient was evident along the nerve (A-E segments). The distribution of enzyme activity in nerves crushed 12 hours earlier appears in Fig. 4 (middle). In the A segment of control nerves proximal to the high crush about 370 per cent of enzyme activity was present. On day 30 the AChE activity of both uncrushed and crushed nerves was not significantly changed from control levels.

The CAT activity of uncrushed control nerves was 750 ± 60 pmol of ACh formed/min/5 mm nerve and no gradient was found along the nerve (A-E segments, Fig. 4). In the A segment proximal to the high crush after 12 hours the amount of CAT activity was about 140 per cent of control. No difference was observed 1, 7 or 30 days after irradiation despite the condition of the irradiated rats.

Discussion

The dose given to the spinal cord (60 Gy) was chosen to enable comparison with the clinical situation in which this dose with certainty would give functional disturbances. Since the volume irradiated was rather small (approximate field size 8 mm \times 3 mm) the dose was comparatively high because the biologic effects of irradiation are known to be related to both dose and volume (cf. discussion in DE SAULT 1958).

The rat was chosen for the experiments for two reasons: the intra-axonal dynamics of rat motor neurons have previously been described (DAHLSTRÖM et al. 1974) and radiation induced symptoms similar to those in humans develop in rats

after irradiation of the spinal cord (INNES & CARSTEN 1961). Thus the rat is a suitable experimental animal for investigating radiation injury (VANDER KOGEL 1977).

No neurologic signs of radiation injury could be observed in rats irradiated 1 or 7 days previously. However, on day 30, 50 per cent of the rats had various degrees of paralysis of the hind limbs, which could be related to the degree of demyelination of the irradiated part of the spinal cord. This observation is in accordance with HOPEWELL & WRIGHT who found that, with an irradiation slit of 8 mm and a dose of 40 Gy, an extensive demyelination of the white matter and neurologic symptoms developed over a period of 1 to 2 months after irradiation. The demyelination was not associated with vascular injury, but was limited to the irradiated region of the spinal cord only.

The process of demyelination had been described by several authors (CARSTEN & ZEMAN, INNES & CARSTEN, ZEMAN & SAMORAJSKI 1971). First, it has been shown that this process does not necessarily precede tissue necrosis (ETSABLE PUIG et coll. 1964, ZEMAN & SAMORAJSKI). Secondly, the influence of the dose rate relationship is of vital importance for the appearance of demyelination (McLAURIN et coll. 1955). Thirdly, it has been emphasized that radiation induced vascular disturbances are of major importance for demyelination as a delayed effect (HAYMAKER). However, this conclusion may not be valid for small fields: signs of vascular injury have not been observed before the appearance of morphologic lesions in the gray and white matter proper (HOPEWELL & WRIGHT, ZEMAN & SAMORAJSKI). In the present experiments demyelination was observed already on day 30, while HOPEWELL & WRIGHT found demyelination to develop between 1 and 2 months. It is possible that the relatively high dose rate used in the present experiments may have hastened the onset of demyelination.

The hind limb paralysis which had developed after 30 days could not be related to changes in the intra-axonal transport of ACh or cholinergic enzymes in the sciatic nerve. In fact, the only two significant changes observed after irradiation were: (1) On day 1 after irradiation (60 Gy) both ACh content of intact nerves and ACh accumulation were higher than in the control group. However, in animals given 200 Gy no change in ACh accumulation occurred, which at present seems to be unexplainable. (2) On day 7

after 60 Gy the AChE activity of uncrushed nerve was about 150 per cent of control. On day 30 irradiation no significant changes in axonal transport were found, neither in severely paralysed rats, nor in animals with mild symptoms. Since the accumulations of various substances proximal to a crush reflect the peripheral synthesis and intra-axonal transport of the enzyme or organelle in question, the results clearly indicate that changes in intra-axonal transport of ACh, CAT or AChE are a major cause in the development of neurologic symptoms following irradiation.

Previously, brain areas were irradiated with higher doses (180 Gy, CATRAVAS & McHALE 1961) which showed that cholinergic enzymes are comparatively resistant to irradiation. In the present experiments the irradiated cell bodies were analysed for activities of CAT or AChE, but minor changes observed in the sciatic nerve are well with these previous observations. However, changes in proteolytic enzymes have been demonstrated by ZEMAN et coll. (1960).

Recently it was demonstrated that transection of the spinal cord at the thoracic level causes a marked alteration in the intra-axonal transport of ACh and AChE in rat sciatic nerve (DAHLSTRÖM et coll. 1978). In the present experiments a severe demyelination of the white matter of the spinal cord did not influence the dynamics of choline substances in the motor neurons in the sciatic nerve. This may suggest that unmyelinated descending fibre systems are more important than myelinated axons for regulating the intra-neuronal dynamics of motor neurons.

SUMMARY

The content and intra-axonal transport of acetylcholine (ACh) and the cholinergic enzymes choline acetyltransferase (CAT) and ACh-esterase (AChE) in sciatic nerve were investigated in rats following single-dose irradiation of the lumbar intumescence of the spinal cord with 60 Gy or 200 Gy. One, 7 or 10 days after irradiation nerve-crush operations were performed, hours before killing and the levels of ACh and enzyme activities in nerve segments relative to the crushes were estimated by biologic (ACh) or chemical (enzyme) methods. The results indicate that alterations in the neuronal dynamics of ACh and related enzymes are a major cause for the development of neurologic symptoms of the motor system after irradiation, and that demyelinated axons are of minor importance for the regulation of cholinergic substances in rat motor nerves.

ACKNOWLEDGEMENTS

Investigation was supported by the Swedish Atomic Energy Council, the Swedish Medical Research Council Nos 04X 7707 04P-4173) and by W and M Vetenskapsfond. The technical assistance of Mrs Johansson and Mrs Kerstin Lundberg is gratefully acknowledged.

For reprints: Dr S Booy, Institute of Neurology, University of Gothenburg, S-400 33 Gothenburg.

REFERENCES

1. A. H. LARSSON B. and REYED B. Zur Morphologie und Strahlenschädigung in Rattenspinalnerv nach Bestrahlung mit 185 MeV Protonen. *Z. Naturforsch.* 60 (1963) 532.
2. O. R. Changes in peripheral nerve tissue after irradiation with high energy protons. *Acta radiol.* 58 (1967) 101.
3. C. and CUTHBERT A. W. A sensitive method for assay of acetylcholine. *J. Pharm. Pharmacol.* 13 (1961) 445.
4. A. and ZEMAN W. The control of variables in autoradiographic studies on mammalian nervous tissue. *J. Radiat. Biol.* 10 (1966) 65.
5. S. G. N. and McHALE C. G. Changed activities of enzymes involved in neurotransmitter metabolism in rats exposed to different qualities of radiation. *J. Neurochem.* 24 (1975) 673.
6. J. A. HEIWall P.-O. BOOJ S. and DAHLÖF. The influence of supraspinal impulse activity on axonal transport of acetylcholine choline acetyltransferase and acetylcholinesterase in rat motor nerve. *Acta physiol. scand.* 103 (1978) 308.
7. J. A. HEIWall P.-O. HEIWall P. O. and ERS N. R. Proximodistal transport of acetylcholine in peripheral cholinergic neurons. In *Dynamics of nerve regeneration and growth in neurons*, ed. by K. Fuxe, L. Olson and Y. Zetterman. Oxford Press, Oxford, New York, 1974.
8. T. I. A. The time-dose relationship in radiation therapy. In *Progress in radiation therapy*, Vol. 1, Edited by F. Buschke. Grune & Stratton, New York, 1968.
9. L. G. J. DE ESTABLE R. I. TOBIAS C. and KER W. Degeneration and regeneration of peripheral nerves in the cerebral and cerebellar cortex following damage from ionizing particle radiation. *Acta radiol.* 4 (1964) 175.
10. Radiochemical microassays for the determination of choline acetyltransferase and acetylcholinesterase. *Biochem. J.* 115 (1969) 465.
11. and GREEN D. P. L. A simple quick microassay for choline acetyltransferase. *J. Neurochem.* 19 (1971) 465.

12. HAYMAKER W. Ohgondendri and rivelin. In *The structure and function of nervous tissue*, Vol. III, p. 468. Edited by G. H. B. Academic Press, New York, London, 1969.
13. HOPEWELL J. W. and WHITE T. A. The effects of dose and field size on late radiation damage to the rat spinal cord. *Int. J. Radiat. Biol.* 4 (1977) 3.
14. INNES J. H. M. and C. K. S. A. Demyelinating or malacic myelopathy. *Arch. Neurol. (Chic.)* 4 (1961) 190.
15. VANDER KOGEL A. J. Radiation damage to the rat spinal cord. Time dose relationship. *Radiology* 127 (1977) 505.
16. LARSSON B. I. E. S. L. P. I. E. B. and SOLRANDE P. Effect of high energy protons on the spinal cord. *Acta radiol.* 51 (1959) 1.
17. LINDER E. Über die funktionelle und morphologische Verhalten peripherer Nerven längere Zeit nach Bestrahlung. *Fortschr. Fortsch. Natur.* 90 (1959) 618.
18. LUBINSKA L. Region of transition between preserved and regenerating parts of myelinated nerve fibres. *J. comp. Neurol.* 113 (1959) 315.
19. MACINTOSH F. C. and PERRY W. L. M. Biological estimation of acetylcholine. *Meth. med. Res.* 3 (1950) 78.
20. McLAURIN R. L. BAILEY O. T. HARSH G. R. and INGRAHAM F. D. The effects of gamma and roentgen radiation on the intact spinal cord of the monkey. *Amer. J. Roentgenol.* 73 (1955) 827.
21. NOTTER G. HALLBERG O. and VIKTERLOF K. J. Strahlenschaden am Plexus brachialis bei Patienten mit Mammakarzinom. *Strahlentherapie* 139 (1970) 538.
22. STOLL B. A. and ANDREWS J. T. Radiation induced peripheral neuropathy. *Brit. med. J.* 1 (1966) 834.
23. TUČEK S. Transport and changes of activity of choline acetyltransferase in the peripheral stump of an interrupted nerve. *Brain Res.* 82 (1974) 249.
24. WESTLING P. SVENSSON H. and HELE P. Cervical plexus lesions following postoperative radiation therapy of mammary carcinoma. *Acta radiol. Ther. Phys. Biol.* 11 (1972) 209.
25. ZEMAN W. and SAMORAJSKI T. Effects of irradiation on the nervous system. In *Pathology of irradiation*, p. 213. Edited by C. C. Berdjus, Williams and Wilkins, Baltimore, 1971.
26. — CARSTEN A. and BIONDO S. Cytochemistry of delayed radionecrosis of the murine spinal cord. In *Response of the nervous system to ionizing radiation*, p. 105. Edited by T. J. Haley and H. Snider. Academic Press, New York, London, 1964.
27. — CURTIS H. J. SCARPELLI D. G. and KLEINFELD R. Chemical and enzymatic changes in nerve cells irradiated with high-energy deuterium microbeams. In *Response of the nervous system to ionizing radiation*, p. 429. Edited by H. Snider. Proc. Int. Symp. North Western Univ. Med. School, Chicago, Ill., September 1960. Academic Press, New York, London, 1960.

THE DEPARTMENT OF TUMOR BIOLOGY II KAROLINSKA INSTITUTET S-10101 STOCKHOLM THE
INSTITUT OF ONCOLOGY UNIVERSITY OF UMEÅ S-90185 UMEÅ AND RADIUMHJEMMET KAROLINSKA
SJUKHUSET S-10401 STOCKHOLM SWEDEN

IRRADIATION COMBINED WITH BLEOMYCIN TREATMENT OF SYNCHRONIZED CELLS IN CULTURE UNDER OXIC AND HYPOXIC CONDITIONS

J. MIDANDER, B. LITTBAND and F. EDSMYR

Materials and Methods

Cells and synchronization procedure A substrain of the Chinese hamster cell line V79 was used propagated under standard tissue culture conditions. The nutrient medium consisted of Eagle's medium in Earle's saline supplemented with 15 per cent fetal calf serum and antibiotics. The cells in the radiation experiments were used after synchronization in their early S or G₂ phase of the cycle.

Synchronization was made by mitotic selection largely according to the method of TERASIMA & TOLMACH (1963). Cells in exponential growth phase were rinsed twice with trypsin buffer, incubated for 15 min and thereafter shaken mechanically for 60 seconds. The procedure was repeated once again and the cells were thereafter incubated for 45 min. After a third final shaking the mitotic cells were collected again. With the method the yield of mitotic cells was 1.5 to 2 per cent of the cell population at start.

The specific phases of the cell cycle were determined in complementary experiments by pulse labelling of the selected cells with ¹²⁵IUdR diluted in trypsin buffer (Amersham) specific activity 37–222 MBq/mg (1–6 mCi/mg). An aliquot of 0.1 ml IUdR solution (37 kBq/1 µCi) was added at one hour

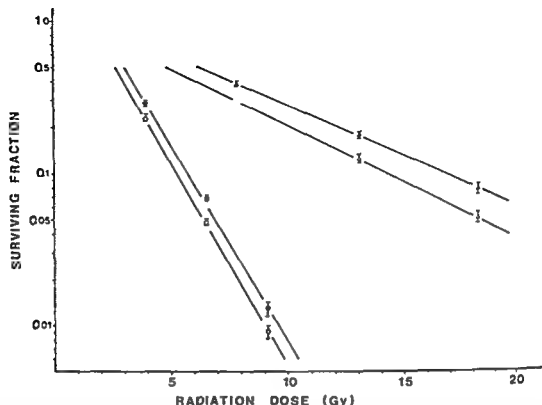


Fig. 1. Radiation survival curves of Chinese hamster cells untreated or pretreated with Bleomycin. The cells were in their early S phase when irradiated. Irradiations were made under oxic (16 paired experiments) or hypoxic conditions in argon (14 paired experiments). Mean values illustrated with SE. The regression

lines were calculated by least square analysis of the pooled data from the comparable experiments. Oxic irradiation with Bleomycin (●), Oxic irradiation without Bleomycin (○), Hypoxic irradiation with Bleomycin (Δ), Hypoxic irradiation without Bleomycin (◊).

intervals during a 13 hour period to each of a series of triplicate Petri dishes (Ø 5 cm) containing the synchronized cells. The labelled cells were incubated for 30 min and the activity was thereafter measured in the washed perchloric acid precipitate of the cells in a well type crystal scintillation counter. The measurements of the relative activity indicated that the early S and G₂ phase followed 3 and 9 hours respectively, the explanation of the selected mitotic cells.

Bleomycin treatment. Bleomycin (H Lundbeck & Co., Copenhagen) when added to the cells was dissolved in the culture medium in a final concentration of 60 µg/ml to which the cells were exposed for 2 hours. As determined in a particular test with asynchronously growing cells, 20±3 per cent of the cell population lost clonogenic capacity due to the cytotoxicity of the substance under these conditions. By pulse labelling with ³HUdR as described the BLM treatment was found to delay progression of cells in the cycle for 15 to 30 min.

Irradiation. The synchronized cells were irradiated 3 or 9 hours after explantation in glass Petri dishes and incubated in the nutrient medium 16

minutes before irradiation the medium drained to an extent that only 0.5 ml was left to cover the cells in order to facilitate gas exchange. The dishes with cover removed were placed in plastic irradiation chamber flushed for 10 min before and during irradiation with either pure oxygen or argon which contained oxygen in a concentration less than 1 ppm. Carbon dioxide was supplied to the gas flow to maintain pH 7.2 in the medium. When BLM was added the cells were exposed to the substance before and during irradiation for a total of 2 hours. After irradiation the cells were washed twice with trypsin buffer and reincubated in fresh medium. Controls were sham irradiated under identical conditions as the irradiated cultures. Reincubation time after irradiation was 8 days, one medium change on the second day. The clones which developed were fixed and stained in situ. Thereafter the mean number of clones was established by counting 3 replicate dishes. The fraction of cells which survived irradiation was expressed as the percentage of the unirradiated controls in the same experiment.

Radiation was generated with a Siemens St

Table 1

Numerical value of the parameters for the survival curves presented in Figs 1 and 2

Cell cycle phase	No of paired replicate experiments	BLM conc (µg/ml)	Plating efficiency (per cent)	Slope constant of survival regression Mean \pm SE (log units/Gy)	D Mean (Gy)	Extrapolation number	
						Mean \pm SE (log units/Gy)	Arithmetic value of mean
Early S	16	80	38 \pm 3	-0.60 \pm 0.019	1.61	0.96 \pm 0.10	2.61
		-	57 \pm 4	-0.600 \pm 0.023	1.67	1.1 \pm 0.13	3.07
G ₂	6	80	27 \pm 3	-0.580 \pm 0.014	1.72	1.10 \pm 0.06	3.01
		-	46 \pm 6	-0.580 \pm 0.018	1.72	1.35 \pm 0.06	3.85
Early S	14	80	49 \pm 4	-0.169 \pm 0.009	5.9*	0.11 \pm 0.11	1.1
		-	64 \pm 5	-0.151 \pm 0.007	6.6*	0.4 \pm 0.07	1.77
G ₂	10	80	33 \pm 4	-0.166 \pm 0.01	6.02	0.74 \pm 0.14	1.27
		-	52 \pm 7	-0.172 \pm 0.01*	6.58	0.16 \pm 0.14	1.17

* Calculated by least square analysis of the survival data in each experiment separately

* Reciprocal of the mean regression coefficient

* Intersection of the regression line with the ordinate at zero Gy

Table 2

Decrease of extrapolation number by Bleomycin treatment for survival curves of the oxically irradiated cells in the experiments presented in Table 1

Decrease of extrapolation number			
Mean \pm SE (log units/Gy)	Arithmetic value of mean	t	p
-0.15 \pm 0.061	0.88	2.05	<0.05
-0.189 \pm 0.063	0.83	2.98	<0.01

Table 3

Increase of regression coefficient by Bleomycin treatment for the survival curves of the hypoxically irradiated cells in the experiments presented in Table 1

Cell cycle phase	Increase of regression coefficient			
	Mean \pm SE (log units/Gy)	Arithmetic value of mean	t	p
Early S	0.037 \pm 0.014	1.03	2.9	<0.05
G ₂	0.013 \pm 0.009	1.01	1.4	>0.1

X-ray Unit at 220 kV and 15 mA. The half layer was 0.3 mm Cu and the dose rate 3.8 Mrads/h at the bottom of the culture dishes. FD=40. The dose delivered was measured by a Philips FD-6 dosimeter.

Results

In a series of experiments the effect of BLM on the radiation sensitivity of Chinese hamster cells in their early S phase was determined by irradiation under oxic (16 replicate experiments) and hypoxic (14 replicate experiments) conditions. The survival was determined in all cases by exposure of the cells to 3 different radiation doses which reduced survival to between 40 and 1

per cent. Fig. 1 illustrates the survival curves calculated by statistical exponential curve fit from the pooled data according to the single hit multi-target model. The curves show that when irradiation was performed under oxic conditions BLM treatment reduced the extrapolation number n and had only a minor effect on D_0 . This can be considered as indicating that the substance interferes with the recovery of the cells from sublethal damage. On the other hand when the irradiation was performed under hypoxic conditions BLM treatment increased the slope of the survival curves i.e. decreased D_0 and had only a slight effect on n . The numerical value of the survival curve parameters are given in Table 1. Statistical analysis (Tables 2 and 3) indicated that the decrease of n under oxic conditions and the

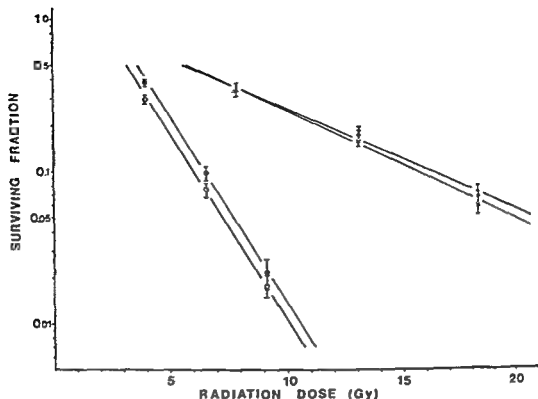


Fig. 1. Radiation survival curves for cells in G phase untreated (○) and treated with BLM (●). Symbols and SE are presented from

26 paired oxic and 10 paired hypoxic experiments. Symbols as in Fig. 1.

increase of the slope of the survival curves under hypoxic conditions are significant.

In other series of experiments the effect of BLM treatment was tested upon G phase cells. As illustrated in Fig. 2 BLM treatment (26 replicate experiments) again decreased the extrapolation number of the cells irradiated under oxic conditions without any effect on D_{10} . In contrast the parameters of the survival curves of the cells irradiated under hypoxic conditions (10 replicate experiments) are not affected by the substance to any significant degree. As indicated in Table 2 the decrease of the extrapolation number of the cells under oxic conditions is again statistically significant.

Discussion

Cell lines of different origin show a great variation in sensitivity to BLM at different concentrations (TWENTYMAN & BLEFHEIN, SAKAMOTO). The cell line used in the present experiments appears to be particularly resistant to BLM. While BLM already at 2 $\mu\text{g}/\text{ml}$ concentration killed 20 per cent of a HeLa cell population (TWENTYMAN & BLEFHEIN), a comparable effect on the Chinese hamster cell line growing asynchronously was found first at a concentra-

tion of 80 $\mu\text{g}/\text{ml}$. As can be calculated from the plating efficiencies indicated in Table 1 the sensitivity of early S phase cells to the cytotoxic effect of BLM is slightly higher (23 and 27 per cent killed under hypoxic and oxic conditions respectively) while the sensitivity of G cells is considerably higher (37 and 41 per cent respectively) than the sensitivity of asynchronously growing cells.

The observation that the extrapolation number of the oxic survival curves of the \square and early phase cells decreased when irradiation was combined with BLM treatment while n of the hypoxic survival curves remained unchanged indicates specific sensitization of the oxically irradiated cells to radiation by interference of BLM with the recovery process from sublethal radiation damage. The data indicate furthermore that recovery is inhibited to a greater extent in the case of \square than early S phase cells. A greater sensitivity of G phase cells to the cytotoxic effect of BLM treatment is also suggested by the lowered plating efficiency of the cells after treatment with the substance and is in agreement with other reports on the specific sensitivity of the cells in this particular phase of the cycle (TERASIMA & UMEZAWA, BARRANCO & HUMPHREY). Interference of BLM with

ation recovery process has been observed previously when asynchronously growing cells were killed under oxic (MATSUZAWA et coll 1972) as well as hypoxic (SHRIEVE & HARRIS 1979) conditions. It can be calculated from the present survival curves that the enhancement factor for the sensitization of early S cells irradiated under oxic conditions is 1.07 and of G₂ cells 1.17 and 1.06 at the 50 and 1 per cent survival level respectively. The interaction between radiation and BLM thus manifests itself most distinctly when relatively small radiation doses are given. Based on this conclusion radiation therapy combined with low doses of Bleomycin has been used in the treatment of malignant tumors in man in a preliminary clinical trial. The preliminary observations (LINDHOLM et coll to be published) agree with the experimental findings that the D₀ of the radiation survival curves was not found to be changed by BLM treatment with the exception of the hypoxically irradiated early S cells. In this case a statistically significant sensitization is present by a dose modifying factor of about 1.1. This value is approximately the same as the one for cells irradiated under oxic conditions due to the argument of the recovery from sublethal damage in the low dose range when they may cancel each other.

In view of the differences in the reaction of G₂ and early S cells the overall result of a combined BLM treatment and irradiation of the heterogeneous asynchronous tumor cell population which grows in vivo depends among other factors upon the particular proportion of oxygenated and hypoxic cells in different phases of the cycle. However, whatever proportions any superiority of the combined treatment in obtaining improved therapeutic results depend upon a specific affinity of BLM to the plastic tissue. Such a specificity has been observed in some cases (CAPUTO 1976) though it may not be a general phenomenon. The sensitization of oxygenated normal tissues may require a decrease of the therapeutic radiation doses in all cases. When tumor specific affinity exists the enhanced sensitivity of the well oxygenated cells in the tumor may be of a particular advantage in the combined treatment. These cells may be killed preferentially leading to an enhanced reoxygenation of the tumor in the hypoxic compartment. Information on the tumor specific affinity of BLM in the individual cases seems to be essential for the design of treatment with the drug in combined modality.

SUMMARY

Bleomycin treated cells are sensitized to radiation delivered under oxic conditions both in the early S and G₂ phases of the cycle. Irradiated under hypoxic conditions sensitization occurs only in the early S phase. This difference in the sensitizing effect of the drug is discussed in regard to the possible clinical advantages of a combined treatment of tumors with irradiation and Bleomycin.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the valuable discussions with Prof. L. Revesz. Skilful technical assistance was provided by Mrs Ingegerd Hedlof and Mrs Rut Jönsson. The investigation was supported by grants from the Swedish Cancer Society, the Swedish Society of Medical Sciences and Wallströms Stiftelse.

REFERENCES

- BARRANCO S. C. and HUMPHREY R. M. The effects of Bleomycin on survival and cell progression in Chinese hamster cells in vitro. *Cancer Res.* 31 (1971) 1218.
- BERDAL P. Head and neck carcinoma. Treatment with Bleomycin and radiation. *Gann Monograph on Cancer Res.* 19 (1976) 133.
- CACHIN Y., JORTAY A., SANCHEZ H., ESCHWEGE F., MADELAINE M., DESAULTY A. and GERARD P. Preliminary results of a randomized EORTC study comparing radiotherapy and concomitant Bleomycin with radiotherapy alone in epidermoid carcinomas of the oropharynx. *Europ. J. Cancer* 13 (1977) 1389.
- CAPUTO A. Importance of experimental data for the improvement of the therapeutic effect of Bleomycin. *Progr. Biochem. Pharmacol.* 11 (1976) 2.
- EDSMYR F. Combined treatment with Bleomycin in penile carcinomas. *Gann Monograph on Cancer Res.* 19 (1976) 231.
- HANSEN H., RYDÅRD J. and ENGELHOLM S. A. Clinical use of combined Bleomycin and radiation therapy for head and neck tumours and testicular cancers. *Bull. Cancer* 63 (1976) 371.
- LINDHOLM C., LITTBAND B. and LÖFROTH P. O. Superfractionated irradiation combined with low doses of Bleomycin in the treatment of oral carcinoma. To be published in *Acta radiol. Oncology*.
- MATSUZAWA T., OZAWA M., MORITA K. and KAKEHI M. Radiosensitization of Bleomycin on lethal effect of mouse cancer cell in vitro. *Strahlentherapie* 144 (1972) 514.
- SAKANOTO K. The effect of Bleomycin and its combined effect with radiation on cultured Chinese hamster cells V 79. *Europ. J. Cancer* 14 (1978) 309.
- SHANTA V. and KRISHNAMURTHI S. The combined therapy of oral cancer. *Gann Monograph on Cancer Res.* 19 (1976) 159.
- SHRIEVE D. C. and HARRIS J. W. Effects of Bleomycin and irradiation on euvic and hypoxic cells. *Int. J. Radiat. Oncol. Biol. Phys.* 5 (1979) 1495.

- SINCLAIR W. K. Cell cycle dependence of the lethal radiation response in mammalian cells. *Curr. Topics Radiat. Res. Q.* 7 (1972) 264.
- SIRACKA E., LITTBRAND B., CLIFTON K. H. and RÉVÉSZ L. Variations in sensitivity of synchronized Chinese hamster cells tooxic and anoxic X ray exposures. *Neoplasma* 22 (1975) 647.
- TERASIMA T. and TOIMACH L. J. Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. *Exp. Cell Res.* 30 (1963) 344.
- UMEZAWA H. Lethal effect of Bleomycin on cultured mammalian cells. *J. Antibiot. (Tokyo)* 23 (1970) 300.
- TAKABE Y. and YASUKAWA M. Combined effect of X ray and Bleomycin on cultured mammalian cells. *Gann* 66 (1975) 701.
- TWENTYMAN P. R. and BLETHMAN M. The sensitivity of cells in exponential and stationary phases of growth to Bleomycin and to 1,3-bis-(2-chloroethyl)-1-nitrosourea. *Brit. J. Cancer* 28 (1973) 500.

NUCLEAR IMAGING OF PULMONARY METASTASES IN THYROID CARCINOMA

A. M. WORM, I. HOLTEN and E. TAANING

Well differentiated thyroid carcinomas (papillary follicular and mixed types) usually have a prolonged course though the incidence of metastases is high. Metastases to the cervical lymph nodes occur in 35 to 80 per cent and distant metastases usually to the lungs in 10 to 40 per cent of the patients (WOOLNER coll 1961 CRILE 1971 LINDAHL 1975 RASMUSSEN 1978). The distribution of metastases differs in the follicular and the papillary carcinomas lymph node metastases being most common in the papillary carcinomas whereas distant metastases occur most frequently in the follicular carcinomas. It has been repeatedly documented that chest radiography does not detect all pulmonary metastases but that scintigraphy with ^{131}I may demonstrate some that are not radiographically detectable (CATZ & STARR 1956 ERNST & HEINE 1961 BARRETT & STENBERG 1965 BONTE & MCCONNELL 1973). Therefore ^{131}I whole body scintigraphy was compared with chest films in a series of patients with well differentiated thyroid carcinoma in order to obtain an increased knowledge of the value of these methods.

Material and Methods

The patients were referred for evaluation and treatment at this oncologic centre. ^{131}I treatment was given to patients with supposed or confirmed tumor tissue outside the thyroid gland.

During a 3 year period (1976 to 1979) 161 whole

body scans were performed in 52 patients (17 males and 35 females mean age 50 years range 14-84 years) with well differentiated thyroid carcinoma diagnosed between 1960 and 1978 but without evidence of co existing malignant disease.

All whole body scans from these 52 patients were reviewed with special reference to abnormal pulmonary ^{131}I accumulation and compared with routine chest films.

Due to varying surgical practice or degree of operability in 20 patients total thyroidectomy was not carried out.

The ^{131}I whole body scintigraphy was performed 2 days after oral $\text{Na } ^{131}\text{I}$ intake using a gamma camera with a whole body imaging accessory (Nuclear Chicago Pho/Gamma III HP IV and a 410 keV parallel collimator). The ^{131}I doses varied from 1.74 to 4.625 GBq (20 to 125 mCi).

Thyroid hormone and dietary iodine intake was avoided for 1 to 2 weeks before the ^{131}I administration. A hypothyroid state was confirmed by laboratory tests including thyroid stimulating hormone (TSH) determination before scanning.

Results

Pulmonary metastases were evident in 14 of the 52 patients diagnosed at the same time as the primary thyroid carcinoma in 7 patients. In 7 patients the

Submitted for publication 1 September 1979

Dr Robert Helig Library

R. M. S. Metastases

lung metastases were detected 5 to 15 years (mean 10 years) after the diagnosis of the thyroid carcinoma.

The lung metastases were demonstrated at scintigraphy as well as at radiography in 7 patients. Four patients had lung metastases demonstrated only at radiography, all with subtotal thyroidectomy.

At whole body scintigraphy 3 patients had abnormal pulmonary ^{131}I uptake, though no abnormality was found at repeat chest radiography within the same period.


Discussion

Some of the decisive factors for the survival of patients with well differentiated thyroid carcinoma are age, histology and the presence of distant metastases (FRANSSILA 1975, MAZZAFERRI et coll 1977, BEIERWALTES 1978). Surgery followed by ^{131}I treatment has reduced the mortality rate (VARMA et coll 1970, HARNESSE et coll 1974, LINDAHL, BEIERWALTES).

As for lung metastases a recent Danish survey of thyroid carcinoma reported a frequency of radiographically demonstrated lung metastases in the well differentiated carcinomas of less than 10 per cent at autopsy of 50 cases. Pulmonary metastases were revealed in 18/8 of these with no abnormality detected on chest films during life (RASMUSSEN).

In the present series lung metastases were evident in a total of 14 of 52 patients at ^{131}I whole body scintigraphy or on chest films. The patient material is selected insofar as the ^{131}I whole body scintigraphy was carried out only in patients with extra-thyroidal tumor tissue. Therefore the number of patients with lung metastases in this series does not reflect the all over frequency of lung metastases in well differentiated thyroid carcinoma. Seven of the 14 patients had lung metastases demonstrated both at radiography and at scintigraphy.

The value of ^{131}I whole body scintigraphy in addition to radiography is demonstrated in the 3 patients with an abnormal scan but negative radiography. These cases are in accord with those of other reports both with respect to the combination of abnormal ^{131}I scintigraphy and normal chest films and to the efficacy of treatment as indicated by the loss of capability of the metastases to accumulate ^{131}I (CATZ & STARR 1967, FRANK & HEINE, BARRETT & STEINBERG, TURNER & WEIR 1972, BONTE & MCCONNELL).



Follicular thyroid carcinoma in a 74-year-old woman. After surgery treated with ^{131}I . a) Whole body scintigraphy after full dose. Diffuse and nodular uptake in the lungs. b) After last dose (total dose 13 875 GBq, 375 mCi) within 6 months: normal scintigraphy. No abnormality detected on repeat chest films.

Four patients had metastases demonstrated at chest radiography. Failure of the tumor to concentrate iodine due to low TSH stimulation could be ruled out as the cause of negative scintigraphy since these patients had laboratory values indicating hypothyroid state with a high endogenous serum concentration of TSH. Subtotal thyroidectomy was performed in these patients, and the initial ^{131}I uptake of the thyroid remnant was of such a degree as to account for the lack of a demonstrable amount of iodine outside the neck (BEIERWALTES). Two of these patients died before further treatment could be instituted.

In the third patient it was not possible to ablate the thyroid remnant despite treatment with ^{131}I to a total of 27.75 GBq (750 mCi). This patient died with widespread metastatic disease.

Although the thyroid remnant was ablated after several ^{131}I treatment doses in the fourth patient, the radiologically evident metastases remained non-demonstrable by nuclear imaging up to the death of the patient 1 1/2 years after the diagnosis was established. Autopsy confirmed the diagnosis.

and carcinoma with pulmonary metastases. The reason why the pulmonary metastases remained undemonstrable at scintigraphy in this patient can be speculated upon.

In order to accumulate ^{131}I in distant metastases, the thyroid gland is totally ablated. This has been described by HARNESSE et al. and CHARBORD (1977). Furthermore PREISMAN & HALPERN (1977) reported that low tumor versus background ratio may camouflage ^{131}I uptake in small pulmonary metastases.

The figure of 14 patients with lung metastases in the present series can only be considered as a minimum incidence. The result clearly demonstrates that neither whole body scintigraphy nor chest radiography alone will reveal all cases of lung metastases of this type of tumor.

SUMMARY

Pulmonary metastases were diagnosed in 14 of 52 patients with well differentiated thyroid carcinoma by whole body scintigraphy or radiography. Three patients had metastases only demonstrable on scintigraphy as chest radiography was diagnostic in 4 patients. Normal scintigraphic findings. Thus, in this type of thyroid carcinoma neither ^{131}I scintigraphy nor routine chest films alone will reveal all cases of pulmonary meta-

For reprints: Dr A. M. Worm, Department of Nuclear Physiology, The Finsen Institute, Strandboulevarden 49, DK-2100 Copenhagen, Denmark.

REFERENCES

- BITO and STEINBERG E. S. Pulmonary metastases from thyroid carcinoma. An unusual case. *Ann intern Med* 67 (1965) 767.
- CHARBORD P. L. and TUBIANA M. The treatment of thyroid carcinoma with radioactive iodine. *Semin nucl Med* 8 (1978) 107.
- CHARBORD P. L., HERITIER C., CUKERSZTEIN W., LUMBROSO J. and TUBIANA M. Radioiodine treatment in differentiated thyroid carcinomas. Treatment of first local recurrences and of bone and lung metastases. *Ann Radiol* 20 (1977) 783.
- CRILE G. Changing end results in patients with papillary carcinoma of the thyroid. *Surg Gynec Obstet* 132 (1971) 460.
- ERNST H. and HEINE H. Schilddrüsenkarzinom. Szintigraphischer Nachweis von Fernmetastasen bei negativem Röntgenbefund. *Fortschr Röntgenstr* 94 (1961) 832.
- FRANSSILA K. O. Prognosis in thyroid carcinoma. *Cancer* 36 (1975) 1138.
- HARNESSE J. K., THOMPSON N. W., Sisson J. C. and BEIERWALTES W. H. Differentiated thyroid carcinomas. Treatment of distant metastases. *Arch Surg* 108 (1974) 410.
- LINDAHL F. Papillary thyroid carcinoma in Denmark 1943-68. II. Treatment and survival. *Acta chir scand* 141 (1975) 504.
- MALAFERRI E. L., YOUNG R. L., OERTEL J. E., KEMMERER W. T. and PAGE C. P. Papillary thyroid carcinoma. The impact of therapy in 576 patients. *Medicine* 56 (1977) 171.
- PREISMAN R. A. and HALPERN S. Detection of metastatic thyroid carcinoma after the administration of a therapeutic dose of ^{131}I iodine. *Europ J nucl Med* 3 (1978) 69.
- RASMUSSEN B. Carcinoma of the thyroid. A survey of 227 cases. *Acta radiol Oncology* 17 (1978) 177.
- TURNER J. E. and WEIR G. J. Pulmonary metastases from thyroid carcinoma detectable only by ^{131}I scan. Treatment and response. *J nucl Med* 13 (1972) 852.
- VARNA V. M., BEIERWALTES W. H., NOFAL M. M., NISHIYAMA H. H. and COLE J. E. Treatment of thyroid cancer. Death rates after surgery and after surgery followed by sodium iodide ^{131}I . *J Amer med Ass* 214 (1970) 1437.
- WOOLNER I. H., BEAHR O. H., BLACK B. M., MCCONAHEY W. M. and KEATING F. H. Classification and prognosis of thyroid carcinoma. A study of 885 cases observed in a thirty year period. *Amer J Surg* 102 (1961) 334.

MICROPROCESSOR SYSTEM FOR TRACKING ISODENSITY LINES
IN FILM DOSIMETRY

R. VAN DER LAARSE and J. DE GANS

The recent fall in price of microprocessors offers interesting possibilities for new approaches in plotting isodensity curves for radiation dosimetry. A cheap, inexpensive equipment is now described which uses an INTEL 8080 microprocessor. The program tracks the isodensity line directly by determining the step length and the step direction from the density measurements.

The tracking procedure is based on the following points:

(1) The length and the direction of each new step is based on the combination of up to the last 10 previous steps. After each step the local density is measured and a new step length and direction is computed.

(2) Isodensity tracking is initiated with a preset starting position, taking the local value as isodensity. Also the direction for the first few steps must be entered if default values are unsuitable.

(3) The step length is adapted to the local density gradient. Density gradients in the central region of the beam are considered as the normal ones. At small depths high dose variations exist at the beam edge; this will produce high density gradients on a small scale. Inside the beam at greater depths and in regions outside the direct beam the corresponding density gradient is low. For steps not coinciding with the mean direction the procedure checks whether the local gradient belongs to the high or low gradient category.

(4) The ragged appearance of isodensity lines caused by small film irregularities, dust particles

etc. is reduced by defining an upper limit between the local signal and the preset isodensity value. Only outside this limit the spot will start to diverge from the mean direction.

(5) The tracking procedure copes with the appearance of film stains (caused by dust particles for instance) of a diameter greater than the step length. If the spot enters a stain it tends to move in a circle inside the stain because it has lost its orientation to the isodensity line. The direction of the last steps are retained to discern this circling.

Equipment

The equipment consists of a densitometer, a moving film table coupled with a drawing board, a fixed pen and a microprocessor (Fig. 1).

Any densitometer can be used which fulfils the following requirements. The light spot on the film should have a diameter of less than 1 mm. The resolution of the densitometer should be about 1% of the full scale with density 1.0, i.e. the lowest density which should give full scale response with maximum amplification. The densitometer signal is fed by means of an AD converter (11 bits) into the microprocessor.

Two stepping motors move the film drawing board assembly in two orthogonal directions. Combining both motors, a step can only be in one of the 8 principal directions (A, Fig. 2). When the equipment

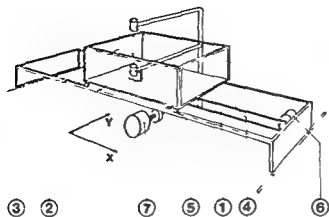


Fig. 1

Fig. 1. Isodensity tracking device. (1) Photomultiplier (2) Lamp housing (3) Film table top (4) Drawing board (5) Drawing pen (6) Stepping motor (7) Stopping motor with high precision thread drive.

Fig. 1 shows the tracking procedure. (A) The eight directions for a step in moving the drawing board in two orthogonal directions. (B) Movement of the measuring light spot is either clockwise (1) or anticlockwise (2) indicated by DRCTN=1 and DRCTN=2 respectively. (C) The inner area of the isodensity indicated by HIGH=1, the outer area by HIGH=0. The system starts the isodensity tracking (START) whenever the film is placed. The default start direction is LR=0. The clockwise and anticlockwise steps are governed by HIGH + DRCTN.

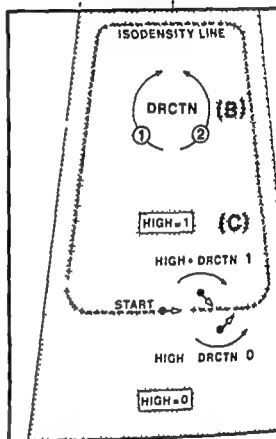
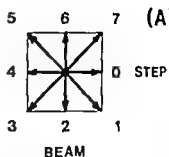
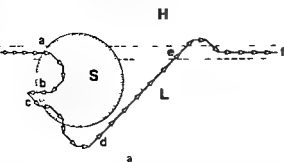


Fig.

is switched on, high precision potentiometers indicate to the processor the table assembly position. All further positioning is made by counting the impulses given to the stepping motors. The drawing board moves under a rigidly fixed drawing pen; the lift mechanism of the pen is controlled by the microprocessor software. A similar mechanical coupling for drawing isodose curves is given by HASKARD (1975).

The microprocessor of the isodensity tracking equipment consists of an INTEL 8080 processor, 6 k bytes Read Only Memory for the software instructions including the tracking procedure and 1 k bytes Random Access Memory for data handling and program stack.

A numerical keyboard is available on the panel to manipulate the film table assembly or to enter a series of data for the film. In the manual mode the table assembly and the corresponding data are displayed by 4 digits. In the automatic mode is used to measure the film and to adjust the isodensity. Next the (x, y) coordinates of the film are entered from which the start position is determined. These position coordinates are used to calculate the percentage depth dose and the metric methods. This approach solves the problem of converting dose.



3 a) Movement of measuring spot with respect to a stain region along isodensity line inside which steps are taken in the mean direction H Region with densities higher than in region I Region with densities lower than in region I S Region with low density a Spot enters high density stain 4 times turning the spot moves in reverse direction (AGAIN=5) b Step in the direction is repeated and the number of these steps stored in ENTER. u Spot leaves stain In this example the isodensity

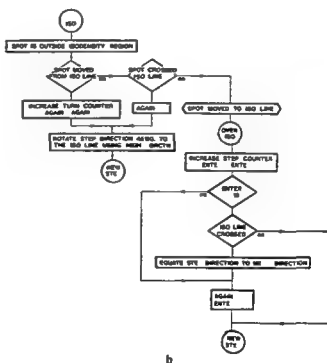
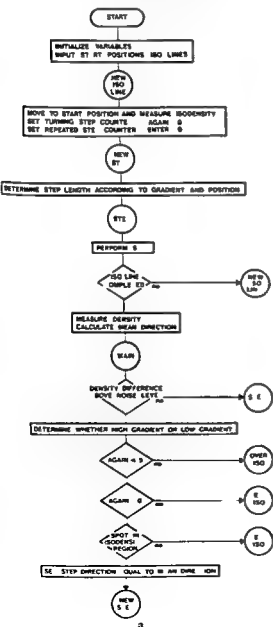
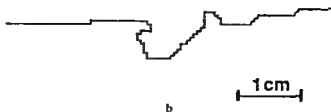


Fig. 4 Flow chart of the tracking procedure

Tracking procedure

The procedure starts with moving the measuring light spot (called spot hereafter) to a position entered manually. The corresponding density is taken as the isodensity value. The pen is lowered and the first few steps are done in either the default direction ($LR=0$, A Fig 2) or in a direction entered manually.

The procedure uses three preset density difference levels. The smallest one prevents the procedure to act on small differences due to noise between subsequent signal values. A second density difference level determines a zone around the isodensity value. If the measured density lies in this zone, the next step will be a step in the mean direction. This zone around the isodensity line will be called the isodensity region.

Within the isodensity region a step is always a 1 mm step in the mean direction. Every time a step outside the isodensity region is taken in a direction not coinciding with the mean direction, the measured density is compared with the previous one. If this local density difference is smaller than the third preset density difference level, the next step will be a 1 mm step. If greater, a step not in the mean direction will be a 0.5 mm step. This step is in the high gradient region.

Let DRC indicate anticlockwise direction and $DRC=1$ the clockwise one (B Fig 2). Movement of the measuring light spot into the inner area is set by variable $HIGH$ to 1 when entering the outer area $HIGH$ is set to 0. When a step moved the spot away from its isodensity value, the necessary change in direction for the next step is given by $HIGH + DRC \cdot FN$ (C Fig 2).

If a previous step moved the spot correctly, the next one is a step in the mean direction of the last 10 steps rounded to the nearest 45°.

Outside the isodensity region, the next step direction will be rotated 45° towards the isodensity line. If the spot then moves towards the isodensity value, this step will be repeated until the isodensity value is reached or passed. However, if the spot moves parallel to or diverges from the isodensity value, the next step direction will be again rotated 45° to the previous one (Fig 3).

After 4 times repeating, this turning the step direction has reversed and the measuring spot tends to move in circles. The variable $AGAIN$ counts the number of consecutively increasing or decreasing density readings. After 4 times $AGAIN$ equals 5 (Fig

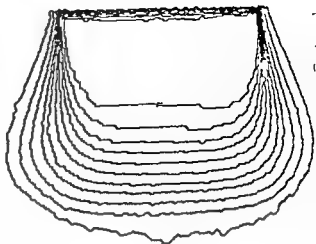


Fig 5. Isodensity curves at 70 MeV electron radiation (field 10 cm \times 10 cm) drawn by the tracking procedure. The isodensity tracking was started on the central axis at the positions of the 90, 80, 10, 5 and 3 per cent depth dose. Note the following characteristics. The beginning and the end of a tracked curve do not necessarily coincide with the 90 per cent line, due to introduction of an isodensity region along the isodensity line. The 3 per cent line shows some meandering in the low gradient region inside the beam.

3). Then all following steps are taken in the last direction. However, if after 10 steps the spot still moves away from the isodensity line, the 45° turning process restarts.

The completion of the isocurve is detected by testing after each step whether or not the spot has entered a small region around the starting position.

More details can be found in the flowchart of the tracking procedure (Fig 4).

Results and Discussion

The tracking procedure has two advantages compared with other techniques. The tracking speed is maximal and a direct coupling with a drawing board is possible, because all steps have a small or zero divergence from the isodensity line. An X-Y recorder is thus not needed, which reduces the cost of the whole equipment substantially.

The combination of global movement and mean direction gives the procedure a remarkable capability to handle irregularities like film stains or isodensity lines starting from a film edge, at the entrance or exit side of the beam. Meandering of the isodensity line occurs only in regions with very low density gradients (Fig 5).

A single set of values for the different density difference levels and the number of last steps is sufficient for the mean direction suffices for all densities.

tested. Since the density differences are all relative to the isodensity value, the accuracy of the density curve obtained is to a high extent independent of the gain of the densitometer, provided the latter is sufficiently high.

SUMMARY

Using a microprocessor a method for plotting isodensity lines in film dosimetry is described. The procedure moves the plotting spot directly along an isodensity line, using the information obtained in preceding steps to determine the next step. For registration of the curves, no X-Y pen recorder

is needed. The essentials of the procedure together with a flowchart are given.

ACKNOWLEDGEMENT

The support of Prof. J. Strackee, Laboratory of Medical Physics, University of Amsterdam, in the development of the tracking procedure is gratefully acknowledged.

REFERENCES

- HASKARD D. L. An automatic X-ray isodose plotter
Phys. Med. Biol. 20 (1975) 645

PHOTON ENERGY SPECTRA OF A HEAVILY FILTERED 300 kV
THERAPY UNIT

T S CHEN K R KASE and B E BJARNGARD

Knowledge of the energy distribution of roentgen beams used in radiation therapy is a useful aid in the design of beam modifying filters for the machine such as flattening and hardening filters as well as wedges and compensators. It is also valuable for detailed analysis of absorbed doses in the patient and in determination of the f factor for bone. During the past decade several reports on measurements of spectra for high energy roentgen machines have appeared (BENTLEY et al 1967, JESSEN 1973, LEVY et al 1974). However, before the recent report on spectral measurements on orthovoltage machines up to 300 kV (SEELENTAG & PANZER 1979) only one seems to have been published (GOODWIN 1966) since bremsstrahlung spectra in this energy range were presented more than 20 years ago (HETTINGER & STARFELT 1958). This sparsity of data no doubt reflects the reduced utilization of orthovoltage roentgen rays in radiation therapy.

The Philips RT 305 machine is designed to produce photon spectra such that the f factor in bone is low, thus allowing irradiation without excessively high doses to bone structures within the irradiated volume (WICHMAN 1965, RADEMACHER & NAGELE 1975). This is achieved by the use of 300 kV acceleration potential and heavy filtration. The intended use is for irradiation at shallow depths and a rapid fall off is assured by the use of short source-to-surface distances (SSD). The treatment cones available have individual filters and produce beam qualities ranging from a 1 mm Cu HVL at 6 cm SSD to a 3.2 mm Cu at 40 cm SSD. The validity of this

design concept is dependent upon the energy spectra relative to the energy dependent absorption in bone. Spectral measurements for this unit seem not to have been reported and are therefore the subject of the present report.

Methods

Spectrometer system The detector chosen for this investigation was a high purity germanium detector (ORTEC Oak Ridge Tennessee) with a sensitive volume 4.25 cm in diameter and 4.76 cm in length. Total energy resolution of the system is about 2.0 keV (full width at half maximum) using 1.33 MeV photons. Detector efficiency relative to a 3 × 3 (7.5 × 7.5 cm) NaI(Tl) detector is approximately 10 per cent for 1.33 MeV photons. The preamplifier and high voltage filter of the system were mounted on the detector which was cooled to liquid nitrogen temperature to reduce thermal noise. The signal was processed by a 4096-channel analyzer and a mini-computer with the flexibility of accumulating and storing data and performing spectrum smoothing. In addition the multichannel analyzer was interfaced to an external computer for further data analysis.

Maximum distance between the roentgen source and detector permitted by the size of the room was somewhat less than 3 m. During normal operation the photon fluence rate was estimated to be about

$10^4 \text{ cm}^{-2} \text{ s}^{-1}$ incident upon the detector at this distance. The maximum count rate for the detector was about 10^4 s^{-1} to avoid distortions due to pulse pile up and dead time. The collimator arrangement shown in Fig. 1 was used to reduce the count rate to a manageable level. Collimator C close to the detector reduces the beam cross sectional area to 1/10014 of the detector cross section. Further reduction was achieved by collimator B which blocks part of the source seen by the detector.

In this manner the count rate was brought below 10^4 s^{-1} . In all measurements dead time losses were less than 15 per cent and the pile up effect was negligible. The measured spectrum of each treatment cone was reproducible from time to time.

Shielding around the detector consisted of a lead cylinder of 2.54 cm wall thickness lined on the inside with 0.32 cm copper to attenuate the lead characteristic roentgen rays. The rear part of the detector connected to the cooling system was covered with lead sheets. In this way the background count rate from scattered photons in the room was reduced substantially.

Experimental techniques The collimator arrangement (Fig. 1) was aligned using a laser beam. First collimators A and D were aligned with the detector and fixed in position. Collimators B and C (both having smaller diameters) were then mounted between collimators A and D on two stands with micrometer adjustments horizontally and vertically. Using the laser beam again collimators B and C were aligned with A and D. Copper was used for collimator C to prevent the generation of lead K roentgen rays and to absorb some lead K rays from the walls of the collimators A and B. In a perfect alignment the lead K lines should be reduced to a minimum in the measured roentgen ray spectrum and this fact was always used for final alignment.

After the detector and the collimators had been aligned the laser was removed and the head of the unit with the treatment cone was placed in position. Since the treatment cone had previously been found to be perpendicular to the tube head levelling the tube head made the central axis of the beam parallel with the horizontal collimation axis. The position of the source was centered on the same line by moving the tube head slightly and finding the position for maximum count rate.

Spectrum measurements were made using four different treatment cones as well as without any treatment cone. The characteristic features of these

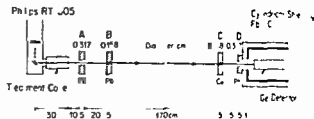


Fig. 1 Experimental arrangement for the spectrum measurements.

Table I
Characteristics of films used

Cone	SSD (cm)	Field size (cm ²)	E ₀ (keV)	Dose rate (Gy/min)
Long A	30	8 × 10	167	1.01
Medium B	15	8 × 10	10	0.91
Medium C	10	6 × 8	40	0.94
Short D	6	3 (dia)	355	0.91

Estimated from half value layer measurements (COHEN et al 1978).

cones are given in Table I. All cones are designed to deliver approximately the same dose rate at constant kV and current. Therefore the shorter cones are more heavily filtered.

Corrections The measured data were corrected for scattered photons generated in the room and entering the detector assembly primarily from the rear. These photons are multiply scattered and their energies are low. Lead of 6 mm thickness was found to be sufficient to prevent most of this radiation from reaching the detector. It was difficult to increase this shielding due to the connections to the liquid nitrogen container. Counts due to remaining scattered photons of this kind were subtracted using a spectrum measured with a lead block in front of collimator D. Scattered photons from the inner walls of the collimators including some characteristic roentgen rays were no doubt present but had been minimized by the careful alignment. The choice of copper for collimator C reduced the presence of characteristic roentgen rays from lead. Further correction was not applied.

Correction for the limited resolution of the detector is unnecessary since it is insignificant in relation to the width of the energy distribution of the bremsstrahlung spectrum. The detector counts include partial absorption events as well as those resulting from total absorption of the photons. The main

PHOTONS/HR RELATIVE SCALE

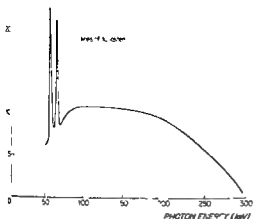


Fig. 2 The roentgen ray spectrum from the RT 305 without treatment cone

PHOTONS/HR NORMALIZED SCALE

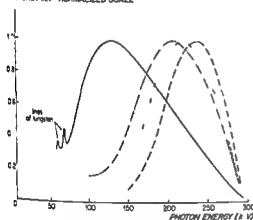


Fig. 3 The roentgen ray spectra from long cone A (—) medium cone B (---) C (— · —) and short cone D (····) The spectra are normalized to 1.0 at maximum

urers specification of the ratio of the photo peak height to the Compton plateau height was approximately 35 to 1 at ^{60}Co and will be even larger at higher energies. Additionally the energies of the Compton edges generally fall below the energies of the main part of the spectrum. However in the lower end of the spectrum particularly without any cone and with the long cone Compton electrons give a small contribution (5–10%). The effect on shapes of the spectra were considered too small to motivate an elaborate correction technique which could have been feasible (LEVY et coll.)

The major correction was that for the energy dependent efficiency of the detector i.e. the fraction of photons incident upon the front surface of the detector that is detected. This efficiency factor

was measured using calibrated nuclide sources with photon energies ranging from 40 keV to 3.25 MeV. The activities of the nuclides were given by the manufacturer (Isotope Products Labs Burbank California) with an average uncertainty of ± 4 per cent. In the photon energy range from 40 to 300 keV the efficiency as defined was found to decrease from 0.75 to 0.34. These measurements were made with a source to detector distance of 25 cm and without collimators. This geometry was different from that used in the bremsstrahlung measurements (Fig. 1). Separate experiments were made with the nuclide sources with and without collimation and the effect on the shape of the efficiency versus energy function was found to be negligible. It should be noted that only the relative efficiency values are of importance and that absolute measurements of photon fluences were not intended. Consequently the measured bremsstrahlung spectra could be corrected with the (arbitrarily normalized) energy efficiency curve obtained with the nuclide sources without collimation.

Results

The corrected roentgen ray spectrum from the RT 305 without any treatment cone is given in Fig. 2. The two K lines of the tungsten target are prominent at the lower end of the bremsstrahlung spectrum and amount to 10 per cent of the total photon fluence. The rather heavy inherent filtration of the tube and its housing (2.5 mm Cu equivalent according to the manufacturer) is obviously attenuating the photons of low energies.

The spectra resulting from the 4 cones specified in Table 1 appear in Fig. 3. The spectra are all normalized to 1.0 at maximum. As can be expected with additional filtration in the treatment cones the peaks of the spectra are pushed to higher energies and the energy range of the spectrum with high filtration becomes narrow. A small amount of tungsten K roentgen rays and some low energy photons below 100 keV can still be seen for the long cone A. The spectra also show that K rays from the lead collimators and shielding have been effectively removed.

Discussion

The fact that correction for partial absorption of the photon energy by Compton scattering was not carried out makes the shape of the spectra at the low

Table 2

Results of experiments and calculations

Cone	Filtration	Measured HVL (mm Cu)	Calculated HVL (mm Cu)	$f_{bone/muscle}$
Long A	4.8 mm Cu	4.0	3.9	1.18
Medium B	1.5 mm Pb	5.2	5.1	1.04
Medium C	2.2 mm Pb	5.8	5.5	1.01
Short D	3.5 mm Pb	6.1	5.7	1.00

energy end somewhat uncertain. Therefore in Figs 2 and 3 the curves were not extended to lower energies than those where the estimated uncertainty is about ± 50 per cent. As discussed previously this effect quickly vanishes at the higher energies and does not affect the main part of the spectrum. Statistical inaccuracies in the spectrum determined by the number of counts per channel were always made negligible ($< 1\%$) by accumulating a sufficient number of counts.

The spectra are representative for the beam axis defined as identical to the collimator axis. No off-axis measurements were made. The use of collimator II (Fig. 1) implies that photons emanating from different portions of the target are not measured with equal efficiency. No effort was made to analyse variations across the focus, but the possible variations cannot be substantial since measurements after independent alignments were reproducible.

As an overall double-check of the spectral measurements the half value layer (HVL) in copper calculated from the spectra in Fig. 3 was compared with that directly measured in a narrow beam geometry (Table 2). From the measured spectrum $N(E)$ the following formula was used to calculate HVL:

$$\frac{D(x)}{D_0} = \frac{\int E \times N(E) \times e^{-\mu x} \times [\mu_{en}/\rho] dE}{\int E \times N(E) (\mu_{en}/\rho) dE} \quad (1)$$

where E is the photon energy, x is the added filtration, μ is the linear attenuation coefficient of a chosen material (in the present experiments copper) and $[\mu_{en}/\rho]$ is the mass energy absorption coefficient for air (used since the measured HVL was based on exposure measurements). Eq. (1) can be solved numerically for $HVL = x_{0.5}$ by setting the dose ratio $D(x_{0.5})/D_0 = 1$.

The calculated HVL are 2 to 6 per cent lower for

in the spectrum measurements (especially for the long cone A) and the difficulty in completely eliminating high energy scattered photons in the HVL measurement (especially for the heavily filtered shorter cones) the results agree more or less as well as can be expected.

The manufacturer describes the RT 305 as a hard roentgen ray therapy unit with a filtration in the range of 5 mm Cu to 3.5 mm Pb designed to achieve water equivalent absorption in bone. In order to confirm this f factor ratios for bone and muscle were calculated:

$$f_{bone/muscle} = \frac{\int [\mu_{en}/\rho]_{bone}^{HVL} \times N(E) dE}{\int [\mu_{en}/\rho]_{muscle}^{HVL} \times N(E) dE} \quad (2)$$

The results are also listed in Table 2. The ratios of the f factors were found to be very close to one for the heavily filtered cones, but are significantly greater for the lightly filtered cones.

Conclusions

For the RT 305 roentgen ray therapy unit the spectra are deliberately changed for different treatment cones which in turn are designed to allow somewhat different but limited treatment depths. The roentgen ray spectrum from the lightly filtered long cone has a maximum at 150 keV and an f factor for bone 18 per cent higher than that for muscle. For the other cones and filtrations the spectra lie primarily above 150 keV with the f factor for bone close to that for muscle.

SUMMARY

Bremsstrahlung spectra from a heavily filtered x-ray therapy unit have been measured using a high purity germanium detector. Measurements were made without filtration and with four different filters: up to 3.5 mm Pb. The photon fluence spectra have maxima at energies ranging from 100 keV to 250 keV. Half value layers calculated from the measured spectra agree within 2 to 6 per cent with measured half value layers. The effective energy absorption coefficient for bone is shown to be within 5 per cent of that for soft tissue for all but the most heavily filtered beam.

ACKNOWLEDGEMENT

This investigation was supported by National Institutes of Health National Research Service Award ES 32606-374 from the National Cancer Institute. The

Wish to thank B. J. Maddox for his help in the calibration of the equipment and the measurements presented at the Annual Scientific Meeting of the American Association of Physicists in Medicine, Atlanta, Georgia, USA, 30 July 1979.

REFERENCES

- ALY R. E., JONES J. C. and LILLICRAP S. C. X-ray spectra from accelerators in the range 2 to 6 MeV. *Phys Med Biol* 12 (1967) 301.
- BEN M., JONES D. E. A. and GREEN D. Central axis depth dose data for use in radiotherapy. *Brit J Radiol* (1978) Suppl. No. 11.
- BRUNN P. N. Spectral measurements of 250 to 300 keV x-rays. *Radiology* 87 (1966) 205.
- HETTINGER G. and STARFELT N. Bremsstrahlung spectra from roentgen tubes. *Acta radiol* 50 (1958) 381.
- JESSEN K. A. Measurements of primary spectra from a kilocurie ^{60}Co unit and a 6 MeV linear accelerator. *Acta radiol Ther Phys Biol* 12 (1973) 561.
- LEVY L. B., WAGGENER R. G., MCDAVID W. B. and PAYNE W. H. Experimental and calculated bremsstrahlung spectra from a 25 MeV linear accelerator and a 19 MeV betatron. *Med Phys* 1 (1974) 62.
- RADEMACHER H. P. and NAGLE E. Intermediate and deep therapy under hard ray therapy conditions. *Medicamundi* 20 (1975) 3.
- SEELENTAG W. W. and PANZER W. Stripping x-ray bremsstrahlung spectra up to 300 kVp on a desk computer. *Phys Med Biol* 24 (1979) 767.
- WICHMAN H. Appropriate radiation qualities in conventional radiotherapy. *Medicamundi* 11 (1965) 61.

SUPERFRACTIONATED IRRADIATION COMBINED WITH LOW DOSES OF BLEOMYCIN IN THE TREATMENT OF ORAL CARCINOMA

C LINDHOLM B LITTBAND and P O LÖFROTH

A combination of irradiation and Bleomycin treatment has been used in the management of many types of tumours with varying results. Thus no effect was encountered in the post-operative treatment of glioblastomas but good results were obtained in the treatment of penile carcinoma (EDSMYR 1976 ANDERSEN et coll 1981). Also in the treatment of head and neck tumours contradictory results have been reported and in some cases severe local reaction of the normal tissue occurred (BERDAL 1976 HANSEN et coll 1976 SHANTA & KRISHNAMURTHI 1976 CACHIN et coll 1977). The results of laboratory experiments also vary indicating differences in the cellular sensitivity to Bleomycin treatment combined with irradiation even in the case of the same cell line when used under different experimental conditions (TWENTYMAN & BLEEHEEN 1973 TERASIMA et coll 1975 SAKAMOTO 1978). At present therefore no general conclusions on the effect of a combination of irradiation and Bleomycin can be drawn. More basic experiments are needed to suggest different regimes to be tested under clinical conditions. However the available data permit some treatment schedules to be defined with a possible control of the local reactions and with possible improved therapeutic results. Thus some preliminary conclusions may be drawn from the present small clinical series treated with a combination of Bleomycin and superfractionated irradiation

Theoretic conditions

Previously the ideas were described which led to the development of a superfractionated regime in the first hand in order to control the radiation re-

sistant hypoxic cells in tumours (JAKOBSSON & LITTBAND 1973 BACKSTROM et coll 1973). This superfractionated regime has now also been used in combination with Bleomycin. The cytostatic effect of Bleomycin has been shown to be biphasic both with regard to time and dose. The survival curve has an upward concave curvature and consists of a steep initial and a less steep terminal portion (URANO et coll 1973 TERASIMA et coll 1976). This indicates that the cells have an early sensitive and a subsequent resistant phase in their response to Bleomycin. However the development of the resistance is time limited and the original sensitivity reappears after 1 to 2 hours. A repeated treatment with small doses after more than 2 hours may therefore result in a better effect than the effect of a single high dose.

A synergistic effect of Bleomycin and irradiation has been demonstrated in different experimental systems. It has been shown that Bleomycin interferes with the radiation recovery processes both in asynchronously and synchronously proliferating cells (MATSUZAWA et coll 1972 SHRIEVE & HARRIS 1979). The interference expresses itself in a decrease of the extrapolation number of the survival curves. The effect of Bleomycin is therefore dependent on the radiation dose being higher after low doses. The results of experiments with synchronized Chinese hamster cells showed an enhancement ratio for G cells of 1.17 and 1.06 at the 50 and 1 per cent survival level respectively. In principle similar results were obtained with cells also in the S-phase (MIDANDER

Table

Treatment schedules and results in 7 cases of oral carcinoma. Classification according to TNM (UICC 1978) and CRE according to et coll (1971). No correction for RBE or volume has been made. Gap correction according to Ellis (1969).

Case No	Age and sex	Site	Size (cm)	Stage	Daily fractions (Gy×times)	Time in interval (hours)	Split course interval (weeks)	Total dose (Gy)	CRE	Bleomycin (mg)		Survival (months)
										Daily dose	Total dose	
1	78 M	Lip	7×1 ⁺	IV	1×3	4 4 16	0	81	19.1	7.5×1	147.5	4+
2	83 M	Lip	3×6	III	1×3	4 4 16	3	80	18.1	7.5×1	195	14
3	74 F	Tongue + lymphog metastases	3×6+	III	1×3	4 4 16	4	75	16.5	5×1	90	48+
4	78 M	Bucca	4×4	III	1×2	6 18	2	78	17.2	7.5×2	173	29+
5	65 F	Soft palate	3×3	II	1×2	6 18	2+2	74	16.1	7.5×2	143	20+
6	70 M	Bucca	4×5	IV	1×2	6 18	2	76.5	17.1	2.5×2	187.5	18+
7	46 M	Tongue	3×6	IV	1×2	6 18	2	77.5	17.1	7.5×2	147.5	1+

Died of pleural empyema

Local recurrence at 4 months

et coll 1980). It may be concluded that a combination of Bleomycin with small radiation doses may be profitable to take advantage of its sensitizing action. This suggests that a superfractionation of the radiation dose in combination with Bleomycin treatment might be of particular advantage.

Material and Methods

The material comprised 7 patients: 5 males and 2 females, between 46 and 83 years (Table). The primary tumour site was the lip, the bucca and the tongue in 2 cases each, and the soft palate in one case, all the tumours being advanced, one tumour in stage II, 3 tumours each in stages III and IV (TNM classification UICC 1978). All tumours were epidermoid carcinomas. The primary tumour and the nearest regional lymph station were included in the target volume. The radiation source was a Brown Boveri Betatron (35 MeV) giving a dose rate of 0.7 to 2.5 Gy/min (SSD 110 cm) and a Varian Linear Accelerator (4 MV) giving 2.0 Gy/min (SSD 80 cm). One patient was treated with a combination of electrons and photons, and the others with electrons only.

The irradiation schedules appear in the Table. Irradiation was given 2 or 3 times daily, in general in 2 equal courses separated by a time interval of 2 to 4 weeks. The total dose was 74 to 81 Gy, CRE=16.1 to 19.1 reu. Bleomycin was given either in 5 mg or 7.5 mg doses daily, one to two hours before the first

irradiation (3 cases) or 2.5 mg twice daily, one to two hours before each irradiation (4 cases). The total Bleomycin dose varied between 145 and 195 mg, with one exception (Case 3) in which the treatment had to be discontinued due to severe sensitivity reaction.

Results

Total remission of all the 7 oral carcinomas occurred in each case before half of the treatment course was completed. During a period of 7 to 14 months (Table) no local or distant recurrence occurred except in one case. One patient (Case 2) died of pleural empyema 14 months after the start of treatment. At post mortem no residual tumour metastases were found, nor Bleomycin-induced fibrosis of the lung tissue. A plastic reconstruction of the lip was made in another patient (Case 1) due to the great loss of tissue as a result of the lip tumour mass. The surgery was performed one month after the completion of the therapy. In the removed tissue no residual tumour was found, and no post-operative complications occurred.

In all patients, confluent mucositis appeared at a dose of about 30 Gy, i.e. some time before the first treatment course was completed. With two exceptions, the mucosal reactions healed during the week rest period, but reappeared before the end of the second treatment course. In one of the cases (Case 3) the local reaction was so intense that the rest period between the two courses had to be

treated while in another case (Case 5) in addition another 2 week rest period had to be inserted after 1 Gy. Within one month after completion of the therapy the local reactions faded away in all cases. During the observation period no osteitis local fibrosis or any complication from the lungs were observed. In Case 7 the locally advanced tumour disappeared completely after treatment. The tumour included the entire tongue and the floor of the mouth. However after 4 months observation a local recurrence in the tongue and a lymph node metastasis occurred.

Discussion

The severe local reaction of the mucosa and other normal tissues has been regarded as the major disadvantage of a combined irradiation and Bleomycin treatment (SHANTA & KRISHNAMURTHI CACHIN et coll.). This reaction can be attributed to the radiation sensitizing effect of Bleomycin on the well oxygenated tissues. The present preliminary results indicate that the severe local reactions can be sufficiently well controlled and avoided to a great extent by superfractionated irradiation combined with Bleomycin treatment in a split course schedule in line with the theoretic considerations. Since the material is still small, no definite conclusions regarding the therapeutic efficiency of this treatment can be drawn. The absence of any severe local reactions may be considered an encouragement to continue this type of treatment. Furthermore, the rapid disappearance of the tumours and the absence of recurrence may be a good prognostic sign particularly in view of the observations of MANTYLA et coll. (1979). The enhanced response may also contribute to a rapid reoxygenation of the tumours (DENKAMP 1977) and thus increase the efficiency of the treatment.

SUMMARY

Seven patients with advanced oral carcinoma have been given 1 Gy per irradiation up to a total dose of 74 to 81 Gy. Bleomycin was given intramuscularly before irradiation. The therapeutic ratio of the proposed scheme was good. The early reactions were severe but were managed by a split course technique. No late complication occurred. All patients were tumour free 18 to 54 months after treatment. Relapse occurred only in one case. The results are not conclusive but bearing in mind that the results in advanced oral carcinoma are poor, they suggest that the present regime may be promising.

ACKNOWLEDGEMENTS

The valuable discussions with Professor L. Revesz are gratefully acknowledged. Certain experiments were supported by grants from the Swedish Cancer Society, the Swedish Society of Medical Sciences and Wallströms Stiftelse.

REFERENCES

- ANDERSEN A P, NESBAKKEN R, HATLEVOLL R, LINDGREN S, NOTTER G, HAGEN E, HOLME I, KOLLE VOLD T, KRISTIANSEN K, TORVIK A, BRUN A, LINDGREN M and ELGEN K. Combined modality therapy of operated astrocytomas grade III and IV. Confirmation of the value of postoperative irradiation. Lack of potentiation of Bleomycin on survival time. A prospective multicenter trial of the Scandinavian Glioblastoma Study Group. To be published in Cancer.
- BERDAL P. Head and neck carcinoma. Treatment with Bleomycin and radiation. Gann Monograph on Cancer Res 19 (1976) 133.
- BACKSTROM A, JAKOBSSON P Å, LITTEBRAND B and WERSALL J. Fractionation scheme with low individual doses in irradiation of carcinoma of the mouth. Acta radiol Ther Phys Biol 12 (1973) 401.
- CACHIN Y, JORTAY A, SANCHEZ H, ESCHWEDE F, MADELAIN M, DESAULTY A and GERARD P. Preliminary results of a randomized EORTC study comparing radiotherapy and concomitant Bleomycin to radiotherapy alone in epidermoid carcinomas of the oropharynx. Europ J Cancer 13 (1977) 1389.
- DENKAMP J. Tumour regression as a guide to prognosis. A study with experimental animals. Brit J Radiol 50 (1977) 271.
- EDSMYR F. Combined treatment with Bleomycin in penile carcinomas. Gann Monograph on Cancer Res 19 (1976) 231.
- ELLIS F. Dose time and fractionations. A clinical hypothesis. Clin Radiol 20 (1969) 1.
- HANSEN H S, RYGD J and ENGELHOLM S A. Clinical use of combined Bleomycin and radiation therapy for head and neck tumours and testicular cancers. Bull Cancer 63 (1976) 371.
- JAKOBSSON P Å and LITTEBRAND B. Fractionation scheme with low individual tumour dose and high total tumour dose. Acta radiol Ther Phys Biol 12 (1973) 337.
- KIRA J, GRAY W M and WATSON E R. Cumulative radiation effect. Part I. Fractionated treatment regimens. Clin Radiol 22 (1971) 145.
- MATSUZAWA T, ONOZAWA M, MORITA K and KAKEHI M. Radiosensitization of Bleomycin on lethal effect of mouse cancer cell in vitro. Strahlentherapie 144 (1972) 614.
- MIDANDER J, LITTEBRAND B and EDSMYR F. Irradiation combined with Bleomycin treatment of synchronized cells in culture underoxic and hypoxic conditions. Acta radiol Oncology 19 (1980) 395.
- MANTYLA M K, KTEKANGAS A E, VALAARAA P.

- and NORDMAN F M. Tumour regression during radiation treatment as a guide to prognosis. *Brit J Radiol* 52 (1979) 972.
- SAKAMOTO K. The effect of Bleomycin and its combined effect with radiation on cultured Chinese hamster cells V 79. *Europ J Cancer* 14 (1978) 309.
- SHANTA V and KRISHNAMURTHI ■. The combined therapy of oral cancer. *Gann Monograph on Cancer Res* 19 (1976) 159.
- SHRIEMI C D and HARRIS J W. Effects of Bleomycin and irradiation on euoxic and hypoxic cells. *Int J Radiat Oncol Biol Phys* 5 (1979) 1495.
- TERASIMA T, TAKABI Y and YASUKAWA M. Combined effect on X ray and Bleomycin on cultured mammary cells. *Gann* 66 (1975) 701.
- WATANABE M, TAKABI Y and MIYAMOTO A. Effect of Bleomycin on proliferative capacity of mammary cells. *Gann Monograph on Cancer Res* 19 (1976) 161.
- TWENTYMAN P R and BILCHINSKY N M. The sensitivity of cells in exponential and stationary phases of growth to Bleomycin and to 1,3-bis (2-chloroethyl) 1 nitrosourea. *Brit J Cancer* 28 (1973) 500.
- URANO M, FUKUDA N and KOIKE ■. The effect of Bleomycin on survival and tumour growth in a mouse mammary carcinoma. *Cancer Res* 33 (1973) 2849.

RADIATION THERAPY OF GLOTTIC CARCINOMA STAGE I

II JOSE D L CALHOUN A MOHAMMED and D A TOBIN

Radiation therapy is being accepted as the primary treatment modality for early carcinoma of the vocal cords. In those patients who fail after radiation therapy cure is possible by surgery. The conservation of voice in this early disease which is frequently achieved with radiation therapy is a very significant point in the management of this disease. In order to achieve optimum results the effects of the total radiation dose, time dose fractionation and the type of radiation must be considered.

Materials and Methods

A detailed retrospective analysis of 321 patients with a diagnosis of laryngeal carcinoma treated at this department from October 1953 to December 1975 was done. Ten patients were excluded because they did not complete the planned treatment. Of the remaining 311 patients 81 (26%) patients were staged as stage I (T1N0M0) glottic carcinoma using the American Joint Committee (AJC) staging system (1978). All the 81 patients were treated with primary radiation therapy with a curative intent and the results are now reported.

The distribution of the patients by age appears in Table 1. The maximum number of patients was in the age range of 45 to 74 years. The sex and race distribution is given in Table 2. Eighty eight per cent of the patients were male and 80 per cent of the total number were white compared with 14 per cent of patients in the black race.

After a complete history, physical examination and review of systems the patients were evaluated

by an otorhinolaryngologist by indirect and direct laryngoscopy. The staging included soft tissue radiography of the neck, tomography and laryngography. All the patients had a microscopic diagnosis of carcinoma. The tumors were classified as squamous cell carcinoma in 69 cases and carcinoma without specification of type in 12 cases.

The major part of radiation treatment was given using megavoltage radiation since the department had a ^{60}Co unit from 1954 and later by 4 MeV linear accelerator. The technique of irradiation was accomplished by using lateral parallel opposed open and wedge fields measuring 4 cm \times 5 cm or 5 cm \times 5 cm. The radiation dose varied from 59 Gy to 68 Gy and the majority of the patients received a midplane dose of 64 Gy. The doses were delivered at a rate of 10 Gy per week in 5 fractions per week.

Results

The survival rates were calculated from the last day of irradiation using the actuarial (life table) method. The crude and adjusted 5 year survival rates are shown in the Figure. In the calculations of the adjusted survival rate those patients who died without disease are withdrawn from the risk of dying from glottic carcinoma. The crude 5 year survival rate was 69 per cent (SE 5%) and the adjusted 5 year survival rate as 86 per cent (SE 4%).

Failures Twelve per cent (10/81) of the patients had local recurrence and were further managed by

surgery or radiation therapy. The clinical data on the 10 recurrences appear in Table 3. The median time of recurrence was 25.5 months. Eight of 10 recurrences (80%) were cured by surgery and the overall local control rate (98%) is given in Table 4.

Complications. No instance of laryngeal necrosis was encountered in the present series. The post irradiation hoarseness and occasional laryngeal edema were controlled in about 2 to 3 months with adequate voice rest and antibiotics.

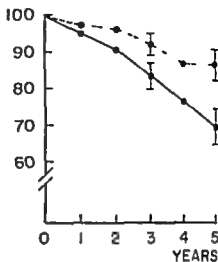
Second malignancies. A second malignant tumor developed in 9 patients: colon 3, lung 2, prostate, hypopharynx, oral cavity, uterine cervix and skin one each. One of these patients had carcinoma of both the colon and the uterine cervix.

Discussion

Several large series are reported on the efficacy of radiation therapy in the management of early glottic carcinoma (JAKOBSSON 1973, JØRGENSEN 1974, HJELM HANSEN et al. 1975, 1979, JØRGENSEN et al. 1979, LUND et al. 1979). Preservation of the voice is an advantage over surgery. In general, the results of radiation therapy have a close relationship to the mobility of the involved cord. WANG (1974) has reported a 5 year recurrence free rate (determinate group excluded those patient dead of intercurrent disease with no evidence of local recurrence) of 87 per cent in a series of 325 patients of stage T1N0M0 with normal mobility. For a tumor limited to the anterior two thirds of the vocal cord, the 5 year NED (no evidence of disease) rate is reported as 92 per cent for lesions of both anterior two thirds and anterior commissure, 81 per cent and for lesions of the entire or posterior third of vocal cord, 70 per cent, respectively. Similar differences in the 5 year NED rate have been reported by SUNG et al. (1979). The 5 year NED rate for carcinoma in situ has been 100 per cent and for T1 lesions it is 76 per cent. HARWOOD et al. (1979) have reported a 5 year crude survival rate of 74 per cent and a corrected actuarial survival rate of 95 per cent in a series of 333 patients.

The superficial carcinoma of the cord is treated up to a dose of 60 Gy in 6 weeks at a rate of 2 Gy per fraction. HORIOT et al. (1972) have recommended 65 Gy in 6 weeks for small exophytic tumors and 70 Gy in 6 weeks for bulky glottic lesions. MORRISON & DELEFY (1962) have reported that 70 Gy in 6 weeks was well tolerated for small size portals (25 cm²)

SURVIVAL



Five year crude (—) and adjusted (---) survival (per cent) rate (life table method).

Table 1
Age distribution at the diagnosis

Age group (years)	No. of patients	Per cent
<45	5	6
45-54	70	75
55-64	24	31
65-74	70	25
>75	11	11
Total	81	100

Table 2
Race and sex distribution

Race	Male	Female	Total	Per cent
White	59	6	65	80
Black	7	4	11	14
Not specified	5	0	5	6
Total	71 (88%)	10 (12%)	81	100

but 64 Gy in 6 weeks was the limit of tolerance for larger portals. In the present series the majority of patients received 64 Gy in 6 weeks which is similar to the dose used by WANG (1974).

Since the lesions have a tendency to spread towards the arytenoid region, a homogeneous distribution of radiation throughout the entire glottic area is required. This can be achieved by using lateral opposed open and wedge fields by an external

Table 3

Failures after irradiation in 10 cases of glottic carcinoma Median time of recurrence was 25.5 months
 DWD Died with disease DI Died with intercurrent disease NED No evidence of disease

Dose (Gy)	Time to recurrence (months)	Treatment	Controlled by surgery	Survival from initial treatment (months)	Status on last follow up
60	8	Laryngectomy+left neck dissection	Yes	46	Died of oral carcinoma
60	10	Laryngectomy+irradiation to stoma (50 Gy)	No	29	DWD
65	11	Laryngectomy	Yes	64	Died of colon carcinoma
65	13	Laryngectomy	Yes	79	DI
6	23	Laryngectomy+irradiation (60 Gy)	No	42	DWD
64	28	Laryngectomy	Yes	84	DI
64	34	Laryngectomy	Yes	77	NED
65	49	Stripping	Yes	97	NED
63	65	Laryngectomy+radical neck dissection	Yes	88	NED
65	75	Laryngectomy	Yes	87	NED

Table 4

Local control rate

	No of patients	Per cent
Primary controlled by irradiation	71/81	88
Recurred	10/81	12
Cured by surgery	8/10	80
Overall local control rate	79/81	98

oblique wedge fields or by a combination of these is described by WANG (1976). The technique of radiation therapy in early glottic carcinoma has also been discussed by MOSS *et coll* (1979). The posterior half of the arytenoid must be excluded from the high dose region by reducing the portals to 50 to 60 Gy in order to prevent excessive arytenoid edema. Also to include the tumor extending to the anterior commissure in the field of radiation the anterior border of the beam should extend one centimeter beyond the anterior edge of the thyroid cartilage.

The reported failure rates in glottic carcinoma Stage I range from 9 to 21 per cent as described by BOSCH *et coll* (1978). In the present series the recurrence rate is 12 per cent and 70 per cent of the recurrence occurred within 3 years after radiation therapy which is similar to the results re-

ported by PEREZ *et coll* (1968) and HARWOOD *et coll*. Both JØRGENSEN & SELL (1971) and NIEDERER *et coll* (1977) have discussed causes of failure including cell type, clinical stage, inadequate size of the portals, etc. Presence of residual malignant cells in the treated volume is the major cause of the failures. The surgical cure rate is high as in the present series (80 per cent). The possibility to cure is dependent on the mobility of the involved cord or the extent of the initial lesion.

The continued follow up of these patients is important to diagnose late recurrences and to find second carcinomas as demonstrated in the present series. HARWOOD *et coll* have reported a second malignancy rate of 2 per cent per year half within the respiratory tract. They had only 4 cases of carcinoma of the larynx and pharynx which did not have an increased incidence of radiation induced carcinomas. Only one case in the present series had a carcinoma of the hypopharynx. These data contradict the argument of using surgery as the primary treatment because of radiation induced carcinoma.

SUMMARY

A detailed retrospective analysis of 81 patients with a diagnosis of glottic carcinoma stage I (T1N0M0) from 1953 to 1975 has been done. Eighty-eight per cent of the patients were male and 116 per cent of the total number

were white. The majority of the patients received a dose of 64 Gy in 6½ weeks. The crude 5 year actuarial survival rate was 69 per cent and the adjusted 5 year actuarial survival rate was 86 per cent. Local recurrence occurred in 12 per cent and of these 80 per cent were cured by surgery. The median time of recurrence was 25.5 months. No case of laryngeal necrosis occurred and 10 second malignant tumors were diagnosed in the follow up.

ACKNOWLEDGEMENT

The authors thank Richard A. Greenberg, Ph.D. and Douglas L. Mills, M.A. for the assistance for the statistical analysis. Philip Lepanto, M.D. and Robert N. Arm, M.D. for the clinical assistance. The investigation was in part supported by Clinical Cancer Education Grant 18006, National Cancer Institute, USA.

REFERENCES

- American Joint Committee for Cancer Staging and end results reporting. Manual for Staging of Cancer p. 39. Whiting Press, Chicago 1978.
- BOSCH A, KADAMIAN M T, FRIAS Z C and CALDWELL W L. Failures after irradiation in early vocal cord cancer. *Laryngoscope* 88 (1978) 2017.
- HARWOOD A R, HAWKINS N V, RIDER W D and BRYCE D P. Radiotherapy of early glottic cancer. I. *Int J Radiat Oncol Biol Phys* 5 (1979) 473.
- HJELM HANSEN M, JØRGENSEN K and SELL A. Carcinoma of the larynx. V. Relationship between biologic effect and failure of irradiation. *Acta radiol Ther Phys Biol* 14 (1975) 305.
- — — ANDERSEN A P and LUND C. Laryngeal carcinoma. II. Analysis of treatment results using the Ellis model. *Acta radiol Oncology* 18 (1979) 385.

- HORIOT J, FLETCHER G H, BALLANTYNE A J and LINDBERG R. Analysis of failures of early vocal cord tumors. *Radiology* 103 (1972) 663.
- JAKOBSSON P. Glottic carcinoma of the larynx. Thesis. Stockholm 1973.
- JØRGENSEN K. Carcinoma of the larynx. III. Therapeutic results. *Acta radiol Ther Phys Biol* 13 (1974) 446.
- — — and SELL A. Carcinoma of the larynx. II. Treatment by ⁶⁰Co supervoltage irradiation. *Acta radiol Ther Phys Biol* 10 (1971) 161.
- — — HJELM HANSEN M, ANDERSEN A P and LUND C. Laryngeal carcinoma. I. Treatment results. *Acta radiol Oncology* 18 (1979) 282.
- LUND C, JØRGENSEN K, HJELM HANSEN M and ANDERSEN A P. Laryngeal carcinoma. III. Treatment results in relation to microscopic score. *Acta radiol Oncology* 18 (1979) 497.
- MORRISON R and DEFFLY T J. The treatment of carcinoma of the larynx by supervoltage radiotherapy. *Clin Radiol* 13 (1962) 145.
- MOSS W T, BRAND W N and BATTISTORA H. *Radiation oncology. Rationale technique results*. Fifth edition p. 216. C V Mosby, St. Louis 1979.
- NIEDERER J, HAWKINS N V, RIDER W D and TILL J E. Failure analysis of radical radiation therapy of supraglottic laryngeal carcinoma. *Int J Radiat Oncol Biol Phys* 2 (1977) 621.
- PÉREZ C A, HOLTZ S, OGURA J H, DEFO H H and POWERS W E. Radiation therapy of early carcinoma of the true vocal cords. *Cancer* 21 (1968) 764.
- SUNG D I, CHANG C H, HARISIADIS L and ROSENSTEIN L M. Primary radiotherapy for carcinoma in situ and early invasive carcinoma of the glottic larynx. *Int J Radiat Oncol Biol Phys* 5 (1979) 467.
- WANG C C. Treatment of glottic carcinoma by megavoltage radiation therapy and results. *Amer J Roentgenol* 120 (1974) 157.
- — — Cancer of the larynx. Radiation therapy. *CA (NY)* 6 (1976) 212.

FROM THE RADIATION THERAPY SERVICE (DIRECTOR Z. PETROVICH) VETERANS ADMINISTRATION
WADSWORTH MEDICAL CENTER LOS ANGELES CALIFORNIA 90073 USA

ADVANCED CARCINOMA OF THE TONSIL

Treatment results

Z. PETROVICH, H. KUISK, L. JOSE, R. BARTON and D. RICE

Carcinoma of the tonsil accounts for over 2 per cent of all malignant tumors and for 10 per cent of those arising in the upper respiratory and digestive tract (TAPLEY et coll 1959). The incidence of carcinoma of the tonsil has increased sharply in the recent years, particularly among patients under the age of fifty (SHUMRICK 1975). This disease predominantly affects males in their sixth and seventh decades of life (SIMONS et coll 1963, SCANLON et coll 1963). In 94 per cent of the patients the tumor is a squamous cell carcinoma (FAYOS & LAMPE 1971), and it does not spread into adjacent structures such as the floor of the tongue and soft palate is common (TERZAGHI 1967, PEREZ et coll 1970).

The incidence of metastatic tumor in the cervical lymph nodes varies from 62 (GARY BOBO et coll 1963) to 75 per cent (FLETCHER & LINDBERG 1966), the ipsilateral subdigastic node being the one most frequently involved. The reported incidence of distant metastases varies from 4 (SCANLON et coll 1963) to 16 per cent (RIDER 1962). The present report reviews the clinical experience with 205 patients with carcinoma of the tonsil who were treated in the Wadsworth Medical Center from 1946 through 1976.

Material and Methods

All 205 patients with microscopically proven squamous cell carcinoma of the tonsil who were treated in this Center are included; no patient with a diagnosis being excluded.

The analysis was accomplished through the review of the records. The initial performance status of the patients was evaluated according to the Karnofsky Scale (KARNOFSKY 1949).

Symptomatology, microscopic appearances, local extent of the tumor, metastatic patterns, treatment methods and results were analyzed. A post mortem examination was obtained in 90 (44%) patients.

Staging. The patients were retroactively staged using the Clinical Staging System for Cancer of the Head and Neck of the American Joint Committee for Cancer Staging and End Results Reporting (1978).

T1 Tumor ≤ 2 cm in greatest diameter

T2 Tumor > 2 cm to 4 cm in greatest diameter

T3 Tumor > 4 cm in greatest diameter

T4 Massive tumor > 4 cm in diameter with invasion of bone, soft tissues of neck or root of tongue

N0 No clinically positive nodes

N1 Single clinically positive homolateral node ≤ 3 cm in diameter

N2 Single clinically positive homolateral node > 3 cm < 6 cm in diameter or multiple homolateral nodes ≤ 6 cm

N3 Massive homolateral node(s), bilateral nodes or contralateral node(s)

M0 No distant metastases

M1 Distant metastases present

Stage I T1 N0 M0

Stage II T2 N0 M0

Stage III T3 N0 M0

T1 or T2 or T3 with N1 M0

Stage IV T4 any T with N2 or N3 M0 and T with any N and M1

Treatment Radiation therapy was the primary treatment in 183 (89%) patients while combined irradiation and surgery was employed in 16 (8%). Combined irradiation and chemotherapy was used on 6 (3%) patients. Before 1969 142 (69%) patients were irradiated with orthovoltage roentgen rays primarily with a beam of the following characteristics: 250 kV, 15 mA, 2.5 mm Cu HVL and a TSD with a minimum of 50 cm. In most of these patients the primary site was irradiated through five portals which included two lateral opposed, one posterior occipital, one submental and a boost field through a transoral cone 3.5 cm in diameter. The lymph nodes of the neck were not irradiated electively but only when palpable lymph nodes were present. The daily dose varied from 150 to 280 cGy. Irradiations were given 5 days a week but not all portals were treated each day. The total tumor dose ranged from 55 to 65 Gy with the mean dose of 60 Gy over a period of 35 to 50 days. In addition 30 of these 142 patients also received irradiation from interstitial implants: ^{226}Ra —7 patients, ^{228}Rn —17 patients, ^{32}P —11 patients (^{32}P was injected into large primary tumors or lymph nodes before 1960).

From 1969 to 1976 63 (31%) patients were given ^{60}Co teletherapy. Opposed lateral portals were used which included the upper cervical lymph nodes. Only a few patients were irradiated through a single ipsilateral portal. The lower cervical lymph nodes were irradiated through a single anterior field which included the supraclavicular region. The patients were irradiated at 80 cm TSD 5 days each week, all portals daily. The daily tumor dose was 200 cGy and a minimum total dose of 60 Gy was delivered over 42 to 50 days. In addition 5 of these 63 patients also received irradiation from interstitial implants: ^{228}Rn —2 patients, ^{192}Ir —1 patient, ^{125}I —2 patients. The lymph nodes of the neck at risk (N0) were irradiated electively.

The 205 patients irradiated included 32 who received less than 50 Gy, of which 16 patients received less than 40 Gy. The reason for these lower doses was palliative treatment in 13/32 pre or post-operative irradiation in 7/32, patients refusal of further irradiation in 5/32, death during the course

Table 1

Multiple malignant primary lesions (except those of the skin and lip) in 51 patients with carcinoma of the tonsil

Site of origin	No of tumors
Lung	9
True vocal cord	8
Floor of mouth	7
Opposite tonsil	7
Anterior 2/3 of tongue	4
Pyramidal sinus	3
Maxillary antrum	3
Soft palate	2
Pancreas	2
Prostate	2
Lymphoma	2
Esophagus	1
Stomach	1
Rectum	1
Carcinoid ileum	1
Total	53

Of the 51 patients 49 had a second primary lesion. Additional 2 had 3 primary malignant lesions except the carcinoma of the tonsil. Thus these 2 patients had 3 separate primary lesions.

of treatment in 4/32 while in 3/32 the reason was not apparent.

Combined surgery and irradiation was employed in 16 (8%) selected patients. Of these 10 were given post-operative radiation therapy (6 for macroscopic disease and 4 for microscopic residual disease) and 6 received preoperative irradiation before radical neck dissection. Combined chemotherapy and irradiation was employed in 6 (3%) selected patients with advanced stage IV tumors. Of these 4 received intraarterial infusion of 5 fluorouracil before the beginning of radiation therapy and 2 received high dose Methotrexate intravenously (50 mg/kg) with Cytarabine Factor rescue. This was repeated once after two weeks.

Patients The age of the patients ranged from 20 to 85 years with the mean age of 59 years. 25 (12%) being under 50 years of age. Of the 205 patients irradiated 202 were males and 3 females. In 40 percent of the patients the initial performance status (IPS) was less than 80 on the Karnofsky scale.

The most frequent pre-existing signs and symptoms were sore throat in 129 (63%) patients, neck mass in 34 (17%) finding of tumor in the routine

Table 2

Incidence of nodal involvement by site in 137 patients

Location	Ipsilateral (n=137)		Contralateral (n=33)	
	No of patients	Per cent	No of patients	Per cent
Subgastric	109	79	27	87
Submaxillary	85	62	23	70
Sublingual	83	60	24	73
Mid posterior	40	30	10	30
Supraclavicular	70	51	9	27
Submental	16	12	6	18
Upper posterior cervical	14	10	8	24
Lower posterior cervical	12	9	5	15
Infraclavicular	3	2	0	0

Table 3

Local extension of the tonsillar carcinoma

Site of extension	No of patients	Per cent
Tonsillar pillars	204	100
Soft palate	191	94
Base of tongue	164	80
Lateral pharyngeal wall	123	60
Retromolar trigone	91	45
Gingiva	77	38
Hypopharynx	71	35
Nasopharynx	67	33
Hard palate	40	20
Floor of mouth	25	12
Mandible	18	9
Anterior 2/3 of tongue	12	6
Larynx	7	3

One patient had disease limited to tonsillar fossa

Table 4

T and N stage distribution in all patients

	T1	T2	T3	T4	Total
N0	8	8	18	34	68
N1	0	4	8	19	31
N2	0	3	10	17	30
N3	0	2	15	59	76
Total	8	17	51	199	205

Includes 1 patient with M1 disease

Includes 4 patients with M1 disease

(24%) had bilateral involvement. Metastatic disease below the clavicle was present in 5 (2%). In 51 (25%) of the 205 patients 53 primary malignancies were detected at sites other than the tonsil. Of these 53 sites 34 (64%) were above the clavicle and 19 (36%) were below the clavicle.

An additional 11 patients had squamous cell carcinomas of the skin or lip and 7 more patients had basal cell carcinoma of the skin. The sites of origin for the multiple primary lesions other than those of the skin and lip are listed in Table 1.

The analysis of the distribution of cervical lymph node metastases (Table 2) demonstrates that the ipsilateral subgastric lymph node was the most frequent site of involvement present in 109 (79%) of the 137 patients who had neck metastases. The submaxillary lymph node was the second in frequency found in 85 (62%) patients. All 33 patients with contralateral cervical lymph node metastases had also ipsilateral nodal involvement.

The microscopic grading of the tumor had the following distribution in 205 patients: Grade I—1 grade II—58 grade III—117 grade IV—27. In two patients the grade of the tumor was not recorded.

Local extension of the tumor was frequent to the tonsillar pillar and soft palate, found in almost all patients (Table 3). Extension to the tongue and lateral pharyngeal wall was next in frequency with 80 and 60 per cent respectively. The distribution of the T and N stage appears in Table 4. It revealed that only 4 per cent of the patients were staged T1, 11 per cent T2, 25 per cent T3 but the majority (63%) presented with advanced stage T4. The N stage distribution shows that 37 per cent of the patients had a massive homolateral, bilateral or contralateral (N3) cervical involvement while 33 per cent had no palpable lymph nodes (N0). The remaining 30 per cent was equally divided between those having a single ipsilateral small node (N1) and those with larger or multiple ipsilateral cervical nodes (N2).

Results

The overall 5 year actuarial survival (Fig. 1) for all patients was 15 per cent with the median survival of 19 months. Detailed analysis of the survival by T and N stage (Figs. 2, 3) showed a progressive decrease of survival with the increase of the T and N stages; however, each of these parameters was acting independently.

The 5 year survival by clinical staging is given in

Physical examination in 21 (10%) trismus in 14 (7%) loss of more than 10 per cent of body weight in 8 (4%) bleeding in one and hoarseness in one. Metastatic disease in lymph nodes of the neck was detected in 137 (67%) patients of which 33

PER CENT

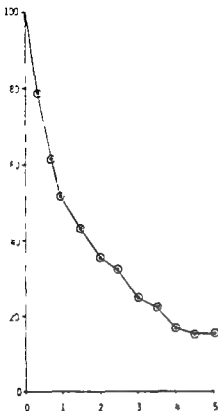


Fig 1

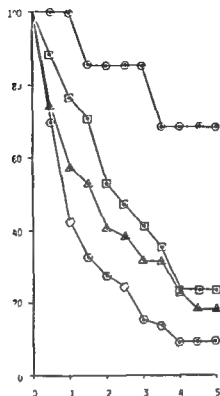


Fig 2

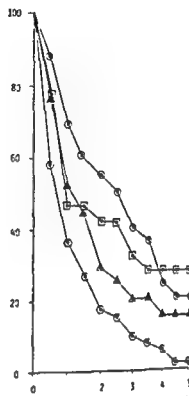


Fig 3

Fig 1 Actuarial survival (per cent) for all 205 patients

Fig 2 Actuarial survival (per cent) by T stage for all patients
● T1 (n=8) □ T2 (n=17) △ T3 (n=51) ○ T4 (n=129)Fig 3 Actuarial survival (per cent) by N stage for all patients
● N0 (n=68) □ N1 (n=31) △ N2 (n=16) ○ N3 (n=6)

Fig 4 It was 69 per cent for stage I and 12 per cent for stage II patients ($p=0.006$). The 5 year survival for stage III patients was 31 per cent. The difference in survival between the stages II and III was not statistically significant ($p=0.1$) due to the small number of patients in stage II. The 5 year survival for stage IV was 9 per cent. The difference in survival of stage III and IV patients was significant ($p=0.02$). The analysis of survival by IPS (Fig 5) showed 22 per cent 5 year survival for patients with IPS >80 against 6 per cent survival for patients with IPS <80 . This difference was significant ($p=0.001$) and it was independent of the stage of the disease.

The analysis of survival by treatment mode (Table 5) showed a 13 per cent 5 year survival for the orthovoltage irradiated patients versus 24 per cent for those given ^{60}Co irradiation ($p=0.12$). The 5 year survival of patients given combined irradiation and surgery was 24 per cent versus 14 per cent for those irradiated only ($p=0.25$).

The survival of the 6 patients given irradiation and

chemotherapy was poor. They had stage IV disease and none of them survived more than 2 years.

The 5 year survival of the 25 patients under 40 years of age was 17 per cent versus 15 per cent for the 180 patients over that age ($p=0.4$).

The current status of all treated patients (Table 6) reveals that a primary tumor control was achieved in 44 (21%) patients. The absence of control at the primary site was responsible for the death of 14 (60%) patients. Metastatic disease with tumor controlled above the clavicle was responsible for the death of 6 (3%) and intercurrent diseases were the cause of death in 18 (9%). Of these 18 patients 1 died without evidence of tumor. Death from malignant disease other than the tonsillar carcinoma occurred in 18 (9%) patients, 8 of whom had no evidence of tonsillar carcinoma. The number of patients lost to follow up was 35 (17%) which included 20 with persistent tumor and 15 with no evidence of tumor.

Metastatic disease below the clavicle (happily or autopsy proven) was found in 44 (21%) patients of

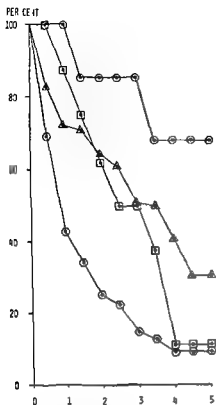


Fig 4 Actuarial survival (per cent) by stage grouping for all patients: \circ stage I (n=8), \square stage II (n=8), \triangle stage III (n=79), \oplus stage IV (n=160).

these 6 had no evidence of tumor above the clavicle.

Detailed analysis was performed of the failure in 68 N0 patients. Among the 52 N0 stage III and IV patients, 17 (17%) had their primary site controlled and did not develop metastases in the cervical lymph nodes.

Seventeen (33%) patients developed a recurrence at the primary site with no evidence of neck node metastases. In 26 (50%) the failure at the primary site coincided with the development of cervical lymph node metastases.

Among the 16 N0 stage I and II patients, 9 (56%) had their primary site controlled and did not develop metastases in the cervical nodes. In 2 (13%) patients the disease recurred at the primary site but there was no evidence of neck node metastases. In 5 (31%) patients the failure at the primary site was followed by cervical metastases.

Surgical cure was attempted in 8 patients who had failed at the primary radiation therapy. In 6 of these the failure occurred in the neck only and in 2 in the neck and the primary site. The surgical attempt to cure was successful in 2 who survived 5 years or

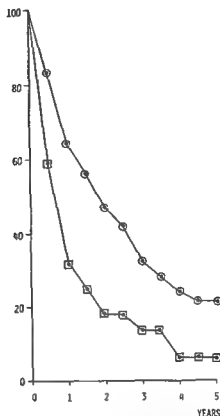


Fig 5 Actuarial survival (per cent) by initial performance status for all patients: \circ >80 (n=127), \square <80 (n=83).

longer. In the remaining 6 patients the tumor recurred shortly after surgery.

Complications. Treatment complications of major importance occurred in 17 (8%) patients. The most frequent major complication was osteonecrosis of the mandible occurring in 14 patients. All of these had an advanced stage IV disease with the tumor invading the mandible. The treatment consisted of conservative management in 12 and surgical resection in 2. Of these 14 patients with osteonecrosis one recovered fully and was free of tumor at 5 years. An additional 3 patients developed post-operative complications: two flap necrosis and one flap necrosis with fistula. These 3 patients subsequently died of massive local tumor recurrence.

Discussion

A series of 205 patients with squamous cell carcinoma of the tonsil was irradiated. The main difference between this series and other reports (RIDER, BALLANTYNE & FLETCHER 1965; PIERQUIN et al 1966; TERZ & FARR, MATAR & MCCARTEN 1973) is the extent of the disease at diagnosis with the present patients having more advanced stages.

RADIATION THERAPY OF NASOPHARYNGEAL CARCINOMA

CHUPIN CHANG TAIFU LIU YUWAN CHANG and SHILONG CAO

Malignant tumors of the nasopharynx are frequent in China, particularly in the coastal provinces south of the Yangtze River, which is in marked contrast to the situation in Europe and North America, where such tumors are rare (CAMMOUN *et coll*).

However, during the years before the Liberation, radiation therapy was used only on a very small number of patients in China, and the results were poor (LIU 1954). Until after 1949, radiation therapy seriously deteriorated. In the following years, irradiation of nasopharyngeal carcinoma was made available to a steadily increasing number of patients. Thus, at this time, a total of 6660 cases of nasopharyngeal carcinoma were treated from 1955 to 1973. In the course of the past 30 years, the authors have had a good opportunity to investigate the radiotherapeutic aspects of carcinoma of the nasopharynx, and mainly on retrospective analyses of some of the cases a few points of special interest are now presented.

Clinical staging

Although the UICC has suggested a TNM classification and clinical staging method for this type of tumor, it has not been universally adopted by workers here and abroad for various reasons. Since the method of staging is intended to relate the extent of the disease to the prognosis after treatment, an analysis of the influence of various clinical factors on survival was performed by CHANG *et coll* (1965), which resulted in the criteria shown in Table 1, which aims to reflect the prognosis more closely than the previously used and thus has been used also in the present report.

The Fifth Chinese National Conference on Nasopharyngeal Cancer at Changsha (1979) suggested some minor modifications in the method of staging mentioned, especially as regards whether a primary tumor less than 0.5 cm in diameter but still involving two walls of the nasopharyngeal cavity should be considered as T1 or T2. The final decision awaits the collective results of a nationwide investigation which is under way.

When metastatic neck nodes are larger than 8 cm in diameter or, although not very large, but appear in the supraclavicular region, there is a direct correlation with the frequency of distant metastasis, which naturally results in poorer prognosis (cf Table 10).

Results of treatment

Each year since Liberation, the 5 year survival rate of nasopharyngeal carcinoma treated by irradiation at this department has risen steadily (Table 2). Although surgery has been advocated intermittently, the results have not been satisfactory. Only very early cases have been found suitable for surgery, and postoperative irradiation was still needed in most of the cases operated upon. As cases of nasopharyngeal carcinoma stage I given radiation therapy alone (Table 2) now have a 5 year survival rate of a good 93 per cent, surgery in its present mode is not indicated as the primary type of treatment.

Of the 838 cases treated from 1955 to 1960, the overall 5 year survival rate for all cases was 27.7 per cent, increasing to 42.8 per cent for the 1914

cases treated from 1961 to 1967 and 54 per cent for the 511 cases treated in 1973 (Table 2)

This gradual improvement may have several causes. The first factor to discuss is whether the relative proportion of early and late cases has changed in the intervening years. Table 3 indicates that no significant change has appeared for the 3263 cases analysed. Cases belonging to stage III and IV have always predominated, being around 75 per cent of all cases treated in the different periods.

Patients with extensive nodal disease rarely survive 5 years after treatment according to WANG & MEYER (1971) nor do patients with erosion of the base of the skull (HOPPE et al. 1976). However, even for such cases in stage IV the 5 year survival rate has increased from 16.5 to 32 per cent in the present series (Table 3).

At present the 5 year survival rate for stage I is 93.3 and for stage II 74.7 per cent. It is thus evident that without any change in the principles of irradiation the overall survival rate was markedly improved simply by detecting and treating the cases of nasopharyngeal carcinoma in their earlier stages. Some progress has been made in this direction recently by the use of serum VCA IgA antibody enzyme labelled immunoassay as part of the clinical examination (LIU et al. 1979). This is often the case when a neck lymph node has been proved by biopsy to be metastatic carcinoma with no apparent primary tumor in the nasopharyngeal cavity. In such instances careful examination of the nasopharynx will sometimes reveal a very small lesion which has been missed at the first examination. Some preliminary results indicate the great value of so-called blind biopsy, i.e. taking a biopsy from the ipsilateral Rosenmüller fossa when a VCA IgA test is strongly positive although no tumor is apparent clinically.

Principles and technique of radiation treatment of nasopharyngeal carcinoma

Radiation source. It is now accepted that ^{60}Co teletherapy units or their equivalent the 4-6 MeV Linear Accelerators are the most suitable machines for irradiation of nasopharyngeal carcinoma. The analysis of the 2752 cases treated from 1955 to 1967 from the viewpoint of the radiation source used (Table 4) substantiates this opinion but some points merit attention.

The better depth dose distribution from ^{60}Co teletherapy units as compared with conventional irradiation was an important factor in improving the

Table 1
Clinical staging for nasopharyngeal carcinoma

Stage	Criteria	TNM
I	Primary tumor limited to one of the nasopharyngeal walls no palpable cervical lymph nodes	T1 N0
II	1 Primary tumor invading two walls 2 Unilateral or bilateral cervical nodes mobile diameter not exceeding 8 cm	T2 N0 N1 (T1 T2)
III	1 Primary tumor extending outside nasopharyngeal cavity into posterior nasal cavity oropharynx, sphenoids, middle ear 2 Cervical nodes partly or completely fixed but diameter less than 8 cm	T3 (N0-N1) N2 (T1 T2)
IV	1 Cranial nerve involvement with or without destruction of base of skull 2 Size of cervical nodes exceeding 8 cm 3 Presence of supraclavicular node 4 Distant metastases	T4 (N0-N1) N3 (T1 T2) N4 (T1 T2) M1

survival rates (Table 4). However, when the dosage by conventional irradiation to the primary tumor had been supplemented by intracavity radium mold treatment the results were much improved. These results corroborate the principle of radiation therapy that adequate dosage has to be concentrated in a small volume of tissue in the region of the primary tumor to achieve long survivals without harmful sequelae. The combination of ^{60}Co teletherapy with intracavity radium in a small group of patients confirmed the value of such a technique in improving the survival rates considerably (Table 5).

A suitable after loading intracavity technique using small ^{60}Co sources is now under consideration which may be expected to further improve the 5 year survival.

However one thing should be noted—the combination of external and intracavity irradiation in stage IV has not improved the results significantly. This may be due to the fact that such cases usually have involvement of the cranial nerves with or without destruction of the base of the skull and the radium mold treatment could not cope with such extensive lesions. Only an adequate tumor dose from external irradiation with careful localization may increase the survival.

Table 2

Five year survival rates for nasopharyngeal carcinoma treated by irradiation in different periods

Stage	1955 to 1960			1961 to 1967			1973 only		
	No of cases	Alive after 5 years	Per cent survival	No of cases	Alive after 5 years	Per cent survival	No of cases	Alive after 5 years	Per cent survival
I	51	33	64.7	75	59	78.7	15	14	93.3
II	168	63	37.5	384	252	65.6	99	74	74.7
III	364	94	25.8	796	329	41.2	278	150	54.0
IV	255	42	16.5	659	172	26.1	119	38	31.9
Total	838	232	27.7	1914	812	42.4	511	276	54.0
			p<0.01			p<0.01			

Most of the cases in this period were given conventional irradiation whereas after 1960 ^{60}Co teletherapy was used for the primary nasopharyngeal tumor

Table 3

Relative proportions of early and late cases in different periods

1955 to 1960		1961 to 1967		1973 only	
No of cases	Per cent	No of cases	Per cent	No of cases	Per cent
51	6.1	75	3.9	15	2.9
168	20.0	384	20.1	99	19.4
364	43.4	796	41.6	278	54.4
255	30.4	659	34.4	119	23.3
838	100.0	1914	100.0	511	100.0

Table 4

Doses of irradiation and 5 year survival rates for all stages between 1955 and 1967

	Total No of cases	Alive	5 year survival (per cent)	
Conventional roentgen (180 kV)	108	67	21.8	
Roentgen rays plus scavintary radium	291	94	32.3	p<0.01
Roentgen ray rotation therapy (200 kV)	107	37	34.6	
Teletherapy	1976	798	40.4	
Teletherapy plus scavintary radium	711	48	68.6	p<0.01

Dosage The question of optimum dosage to the primary nasopharyngeal tumor should be viewed not only from the standpoint of years of survival but also from that of the condition of health of the surviving patients. Since most of the patients with stage I and II surviving for more than 5 years have a very good chance also to survive for over 10 years the problem of late radiation sequelae should be kept in mind when treating these patients. At present some authors consider that in order to obtain complete eradication of a tumor irradiation should be carried out to the limit of tolerance of the adjacent normal tissues. However the opinion at this hospital is that pushing normal tissue dosage to the limit of tolerance is of doubtful value in increasing long term survival rates while the incidence of serious sequelae is inevitably augmented. Table 6 indicates that for most cases a dose of 55 to 60 Gy in 6 weeks is quite sufficient to control the primary tumor and a higher dose does not increase the survival rate significantly.

The type of the nasopharyngeal malignant tumor has some influence on the dose that should be given. For anaplastic carcinoma, lymphoepithelioma and poorly differentiated squamous cell carcinoma constituting 85 per cent of the present cases a dose of 50 to 60 Gy is quite adequate. The more infrequent well differentiated squamous cell carcinoma may need a slightly higher dose 62 to 65 Gy and some adenocarcinomas may be very resistant to irradiation. Also large bulky tumors would require the higher dosage.

Table 5

Combination of ^{60}Co teletherapy and intracavitary radium in the treatment of nasopharyngeal carcinoma (1961-1967)

Stage	No. of cases	Alive	5 year survival (per cent)
I	9	9	100.0
II	26	0	76.9
III	8	17	60.7
IV	7	2	28.6
Total	70	48	68.6

Table 6

Relationship of dosage to 5 year survival rates in nasopharyngeal carcinoma (1955-1967). Sixteen cases whose radiation schedule was irregular have not been included

	Dose (Gy)			
	30-	40-	50-	60-
No. of cases	36 ^a	259	1,008	1,107
Alive	94	87	406	470
5 year survival (per cent)	27.1	31.7	40.3	40.7

Table 7

Subsequent occurrence of cervical metastases in 140 cases with and without elective irradiation of the neck (1955-1967)

Size of primary tumor	Elective irradiation omitted			Elective irradiation given		
	No. of cases	Subsequent cervical node metastases	Per cent	No. of cases	Subsequent cervical node metastases	Per cent
T1	15	0	0	111	4	3.6
T2	36	8	22.2	149	11	7.4
T3	7	2	28.6	23	1	4.3
T4	36	6	16.7	64	3	3.1
Total	94	16	17.0	347	18	5.2

$p < 0.005$

Technique of irradiation The total dose to the primary tumor is given through two opposed preauricular portals (receiving 2/3 to 4/5 of the total dose) and two angulated malr portals (receiving 1/3 to 1/5 of the total dose). The former usually measure 5 cm × 7 cm or 5 cm × 6 cm and the latter 4 cm × 5 cm. The size of the preauricular fields is enlarged forwards to 6 cm width if the tumor has invaded the posterior nasal orifices and downwards to 8 to 9 cm if the tumor extends down to the oropharynx. The two malr portals are replaced by a frontal nasal portal or by more angulation inwardly of the beam to the malr portals in the case of invasion of the posterior nasal cavity. A posteroauricular portal may sometimes be used for cases with cranial nerve involvement or erosion of the base of the skull.

In contrast to metastatic neck nodes of other kinds of head and neck carcinomas it has been shown that it is possible to cure those of nasopharyngeal carcinoma with irradiation. The entire chains of lymph nodes of both sides of the neck were first irradiated by ^{60}Co through tangential frontal

fields followed by direct conventional irradiation. The total dose to the involved side of the neck amounted to approximately 50 Gy in 6 weeks.

It should be emphasized that elective irradiation to all cervical regions not involved clinically (N0 cases) should be considered. Table 7 indicates the usefulness of such irradiation especially in the cases with large primary tumors (T3). The probability of a metastatic node appearing later is much lowered when elective irradiation has been given. A tissue dose of 35 Gy was usually given in such cases.

Cause of death

An analysis of the causes of death in patients treated by irradiation is of value as a guide to further improvement of the survival rate. Table 8 shows the known causes of death in 187 of the 735 cases who died within 5 years after treatment given in 1973.

It is apparent that distant metastasis predominates as the cause of death. The rate of distant metastases

Table II

*of death following irradiation of nasopharyngeal car
(1973) Forty eight cases whose cause of death is un
known have been excluded*

	No of cases	Per cent
currence of primary tumor	45	24.1
nce in the neck	20	10.7
metastases	99	52.9
primary tumor	4	2.1
in myelitis	3	1.6
debility	10	5.4
iceros causes	6	3.2
	187	100.0

Table 9

*ship between extent of primary tumor and incidence of
distant metastases in 110 patients (1973)*

	Extent of primary tumor				Total
	T1	T2	T3	T4	
cases	15	47	39	26	127
istant ases	0	1	6	4	11
t	0	2.1	15.4	15.4	8.7

Table 10

*ship between size of cervical nodes and incidence of
metastases in patients with nasopharyngeal carcinoma
(1973)*

	Diameter					Total
	None	1 cm	3 cm	5 cm	8 cm	
cases	127	98	131	120	35	511
istant itases	11	19	26	33	14	103
nt	8.7	19.4	19.8	27.5	40.0	20.2

5

(%) in the cases who died is much higher than reported by CHEN (1971) but similar to that in Kwangtung Province (45.5% Chung San ical College 1974) At autopsy TEOH (1957) d 15 cases of distant metastases (48.4%) in 31 s with nasopharyngeal carcinoma The preven of distant metastases in nasopharyngeal car

cinoma as in other malignant tumors is thus an important but difficult problem that urgently needs to be solved Theoretically routine adjuvant chemotherapy after irradiation may be of some effect but that awaits confirmation by a controlled clinical trial in a large number of patients The present material shows that the most important measure to be taken is as expected early diagnosis followed by immediate treatment If the primary tumor as well as its neck metastases are treated in their earlier stages the incidence of distant metas tases can be greatly reduced (Tables 9-10) thereby increasing the survival rate

Comparison of Tables 9 and 10 shows that the presence and especially the size of the cervical nodes is more important than the extent of the primary lesion as regards the incidence of distant metastases occurring after irradiation BERGER & FLETCHER (1971) came to the same conclusion

The second important cause of death is local recurrence This is perhaps due more to geographic miss than to general underdosage as recurrence in the nasopharyngeal cavity often occurs at a site dif ferent from the original one However some naso pharyngeal tumors have shown an unexpected resistance to radiation a small but marked residual mass may remain after full dose external irradiation In such cases a further boosting dose may be required to eradicate the residual tumor This is best given using intracavitary radium local excision by surgery may also be considered instead At tempts to increase the external irradiation to a dose of 70 Gy or more will increase the incidence of late complications

The third and less important cause of death is due to recurrence of the neck nodes after irradiation Although not often occurring it is a rather difficult situation as this kind of recurrence usually occurs in a tumor bed markedly altered by radiation fibrosis with atrophic overlying skin

Concluding remarks

The results gained at this hospital with radiation therapy of malignant nasopharyngeal tumors one of the most frequent malignant tumors in China indicate that all possible efforts should be made to improve the methods for early diagnosis As soon as the diagnosis has been established irradiation should be given as the primary treatment

SUMMARY

Carcinoma of the nasopharynx is one of the most frequent malignant tumors in the southern parts of China. A total of 3263 cases treated with irradiation in the course of the thirty years since the Liberation have been analysed retrospectively. The 5 year survival rate has risen from 27.7 per cent for cases treated during 1955 to 1960 to 54 per cent for those treated in 1973. The technique of irradiation is described emphasizing the importance of suitable techniques for the treatment of the cervical lymph nodes. The value of elective irradiation of cervical regions not involved clinically is elucidated. Finally the causes of failure of treatment are discussed.

REFERENCES

- BERGER D S and FLETCHER G H Distant metastasis following local control of squamous cell carcinoma of the nasopharynx, tonsillar fossa and base of tongue. *Radiology* 100 (1971) 141.
- CAMMOUN M, HOERNER G V and MOURFLIN Tumours of the nasopharynx in Tunisia. *Cancer* 33 (1974) 184.
- CHANG C P, LIU T F and YU E X A clinical staging method for carcinoma of the nasopharynx. *Chin J Radiol* 10 (1965) 457.
- CHEN E Y Malignant tumors of the nasopharynx. *Radiology* 99 (1971) 165.
- Chung San Medical College Dept of Radiology Tumor Hospital Radiotherapy of nasopharyngeal cancer. *Chin med J* 54 (1974) 687.
- HOLLIF T, GOFFINET D H and BAGSHAW M A Carcinoma of the nasopharynx. *Cancer* 37 (1976) 2605.
- LIU S L, JIANG J Q, HU X J, FU X Z, LI X M and YU H Immunoassay of IgA VCA in serum of nasopharyngeal cancer patients. *Chin J Oto Rhinolaryng* 14 (1979) 209.
- LIU T F Clinical analysis of malignant tumors of the nasopharynx. *Chin J Radiol* 1 (1954) 31.
- TIEN T H Epidermoid carcinoma of the nasopharynx among Chinese. A study of 31 necropsies. *J Pathol Bact* 73 (1957) 451.
- WANG C C and MEYER J E Radiotherapeutic management of carcinoma of the nasopharynx. *Cancer* 78 (1971) 566.

FROM THE DEPARTMENT OF RADIATION ONCOLOGY UNIVERSITY OF WASHINGTON HOSPITAL SEATTLE WASHINGTON 98195 AND NORTHWEST CONJOINT RADIATION ONCOLOGY CENTER AND THE DEPARTMENT OF RADIOLOGICAL SCIENCES CENTER FOR THE HEALTH SCIENCES LOS ANGELES CALIFORNIA 9024 USA

FAST NEUTRON TELETHERAPY FOR ADVANCED CARCINOMA OF HYPOPHARYNX AND SUPRAGLOTTIC LARYNX

G E LARAMORE J JOHNSON T W GRIFFIN D TONG M T GROUDINE
J M KURTZ A H RUSSELL and R G PARKER

In selecting a therapeutic approach to the treatment of carcinoma of the hypopharynx and larynx consideration must be given to the preservation of voice and the minimization of aspiration problems. In many centers definitive radiation therapy is the treatment of choice for early lesions and excellent rates of local control have been achieved (GOFFINET et coll 1973 WANG 1973 GOEPFERT et coll 1975 NASS et coll 1976 HARWOOD et coll 1979). However, more advanced lesions are difficult to control with either conventional photon radiation alone (GOFFINET et coll WANG INOUE & SHIGEATSU 1976 NASS et coll) or in combination with surgery (GOEPFERT et coll MARKS et coll 1978). Some authors advocate using higher total doses of conventional radiation (KARIM et coll 1978 STRYKER et coll 1979) while others (HARWOOD & FIERIE 1979) show that the risk of major complications increases once an NSD of 2050 ret is achieved and that at least for early glottic lesions the dose response rate is fairly flat above 1650 ret. The difficulty in achieving good local control using conventional photon radiation for large tumor masses may in part be due to a significant hypoxic cell population which is fairly resistant to low linear energy transfer (LET) modalities. This accounts for the current interest in high electron affinity radiation sensitizers such as misonidazole and in the high LET

radiation modalities such as neutrons, pions and heavy ions (WITHERS 1973).

The results of a Phase I preliminary trial at this University are now reported in which fast neutrons from a cyclotron designed for physics research purposes were used to treat patients with advanced squamous cell carcinoma of the hypopharynx and supraglottic larynx. This work is part of a larger test of the use of fast neutron radiation therapy for advanced carcinoma of the head and neck region. The present report completes a site by site analysis of this work with the results for the oropharynx (GRIFFIN et coll 1979 LARAMORE et coll 1979) and the oral cavity and soft palate (LARAMORE et coll 1970) having been reported elsewhere. The majority of the patients in the present series had advanced cervical adenopathy as well as large primary lesions but here only the control at the primary site will be considered. The control of advanced cervical adenopathy with fast neutron radiation has been described elsewhere (GRIFFIN et coll 1978).

Methods

The patients were treated with fast neutrons produced with the University of Washington physics

cyclotron. The beam characteristics of this machine have been described in considerable detail elsewhere (WOOTTON et al. 1975, WEAVER et al. 1977) and so will be only summarized here. The neutron beam is produced by accelerating deuterons to ~ 22 MeV and then impacting them on a beryllium target. This produces a relatively broad energy distribution with the mean energy being ~ 8 MeV.

Only a single horizontal port was available for biomedical work and so the patients were treated in a specially designed chair which could be accurately positioned in front of the port. Individual treatment fields were shaped using collimators made from a water impregnated plastic. A photon contaminant in the beam is caused by neutron nuclear interactions and this varies with target depth (WEAVER et al.). It is less than 10 per cent at a depth of 10 cm but is implicitly included in the term cGy_{N} (rad_{N}). Patients were treated at a source skin distance of 150 cm and at this distance the treatment dose rate was ~ 40 $\text{cGy}_{\text{N}}/\text{min}$. For typical field sizes used in the present work the 50 per cent isodose line in a tissue equivalent phantom lies at a depth of ~ 9 cm at beam center. Hence the neutron beam is somewhat less penetrating than ^{60}Co radiation.

Before beginning clinical trials the relative biological effectiveness (RBE) of the beam compared with ^{60}Co radiation was estimated using various animal models (GERACI et al. 1974, 1975, NELSON et al. 1975). The actual RBE obtained depended on the fractionation scheme, the tissue type and the endpoint chosen. Based upon the available data at the time of the clinical trials an RBE = 3 was chosen for use in setting the original neutron dose levels and in relating dosages to photon dosages previously used for comparable tumors. The original animal experiments used relatively small numbers of fractions and based upon the reduced probability for repair using high LET radiation a much smaller shoulder on the cell survival curve, the more highly fractionated schemes could correspond to higher RBE values. Another point that is obvious in retrospect is that since the neutron beam deposits about 85 per cent of its energy via a knock-on process where a fast neutron transfers a large fraction of its energy to a proton the energy deposition process would be more efficient in tissues with a high hydrogen content such as adipose and neural tissue. Using the calculated energy deposition in muscle tissue as a baseline clinical experiences thus far

point to an RBE >3 for a late neural tissue damage (LARAMORE et al. 1978, 1979) but no unexpected complications occurred in the present work.

Various treatment fractionation schemes were used in keeping with somewhat limited access to the cyclotron for medical purposes. In an attempt to standardize these schemes 300 cGy_{N} (about 9 photon cGy equivalent) per week were given each. Three treatment schedules were investigated for treatments utilizing neutrons alone: (1) 1 cGy_{N} on Mondays and Fridays; (2) 100 cGy_{N} on Mondays–Wednesdays–Fridays; and (3) 75 cGy_{N} on Mondays–Tuesdays–Thursdays–Fridays. At first patients were arbitrarily assigned to either the 1 or 3 neutron fractions per week schedule but later the 4 neutron fraction per week schedule was adopted as standard by all neutron programs in the United States. In the present series as well as in others from this institution (GRIFFIN et al. 1977, 1979, LARAMORE et al. 1978, 1979, 1980) no difference among these schemes was found either in terms of local control rate or in terms of adverse effects. Laboratory examinations at this institution by NELSON et al. and RASFY et al. (1977) had suggested an enhanced therapeutic ratio for 2 neutron fractions plus 3 photon fractions per week mixed beam (neutron/photon) treatment arm was added in order to test this in a clinical setting in which 60 cGy_{N} were given on Mondays–Fridays and 180 cGy_{N} of ^{60}Co radiation were given on Tuesdays–Wednesdays–Thursdays. Patients were treated using a shrinking field technique as described by FLETCHER (1973). The spinal cord dose was limited to <1500 cGy_{N} on the neutron only regimes and <600 cGy_{N} + 2700 cGy_{N} on the mixed beam regime. In general the primary and any area of massive adenopathy received between 1700 and 2700 cGy_{N} on the neutron-only regimes and between 840 and 900 cGy_{N} + 3780 to 4140 cGy_{N} on the mixed beam regime. In the early phase of the trial 3 patients with laryngeal tumor and 3 patients with hypopharyngeal tumor who were treated with neutrons alone did not receive radiation therapy to clinically uninvolved lower neck and supraclavicular areas while 2 patients with hypopharyngeal tumor received prophylactic neutron radiation to these areas. The remainder of the patients received between 4500 and 5000 cGy_{N} ^{60}Co radiation to these regions using a single anterior field. In general a small midline block was used in the anterior field at its junction with the upper neck fields. However

this would have compromised the treatment then midline block was used and the field junction was moved every 2 weeks. The treatment fields were set using either a Northwest Medical Physics Cassegrain simulator or a General Electric orthovoltage unit and confirmed with beam films taken on the cyclotron or megavoltage treatment unit. Patients receiving photon radiation were treated in the usual supine position while the neutron radiation was delivered with the patient sitting as mentioned.

Material

Hypopharyngeal tumors: The usual convention of defining the hypopharynx as extending from the plane of the hyoid bone to the plane of the lower border of the cricoid cartilage was followed. It includes the pyriform sinuses, the posterior cricoid area, and the lower portion of the posterior pharyngeal wall. Twenty patients with advanced hypopharyngeal tumor were treated with fast neutrons during the period from October 1973 through August 1977. Of these, one patient received preoperative mixed beam (neutron/photon) radiation of 3060 cGy, + 600 cGy, ~ 4860 cGy equivalent; one received 1450 cGy_n, neutron radiation; one received postoperative mixed beam irradiation of 2880 cGy, + 540 cGy_n, ~ 4500 cGy equivalent; one patient received a 600 cGy_n neutron boost to the primary after receiving 5040 cGy, megavoltage photon therapy. The patient who received preoperative mixed beam radiation therapy had excellent tumor regression and only carcinoma in situ was found in the operative specimen. She died disease free at the primary site 16 months after the operation but had also developed pulmonary metastases. The patient who received preoperative neutron only radiation died disease free 15.5 months after surgery but in the immediate postoperative period developed a pharyngeal cutaneous fistula. The patient receiving postoperative mixed beam radiation developed a pharyngeal cutaneous fistula and died 10 days after completing radiation therapy. The patient who received the neutron boost was thought to have had complete regression of his primary and neck nodes (T4N3) after completing treatment but then developed a local recurrence 3 months afterwards. He underwent a salvage surgical procedure but had tumor present at the surgical margins and developed a second clinical recurrence 4 months after operation. He died of progressing

tumor one year after completing the irradiation. The complications of combined surgery and neutron radiation for head and neck carcinoma have been discussed in detail by GRIFFIN et al (1979) and will not be extensively dealt with here. These 4 patients do not fit into the scope of the present analysis and will not be considered further. This leaves 16 patients with hypopharyngeal tumor treated for cure with neutron or mixed beam teletherapy who constitute the material for this phase of the investigation.

Of these 16 patients, 10 were treated with neutrons alone and 6 were treated with the mixed beam regime. For the mixed beam group, the mean age was 60.1 years (range 59-69) with the male:female ratio being 5:1 while for the neutron-only group the mean age was 64.3 years (range 45-75) with the male:female ratio being 8:2. In almost every case, the patients presented with a significant history of ethanol and tobacco abuse. All patients underwent a direct endoscopic examination under anesthesia and had biopsy proven squamous cell carcinoma. In the neutron only group, one tumor was well differentiated, 6 tumors were moderately differentiated and 3 were poorly differentiated. In the mixed beam group, 5 tumors were moderately differentiated and one tumor was poorly differentiated. The lesions were staged according to the recommendations of the American Joint Committee for Cancer Staging and End Result Reporting, which is shown in abbreviated form in Table 1. The distribution of hypopharyngeal lesions according to this staging system appears in Table 2. It is important to note the advanced primary and nodal disease status of the patients. Such an advanced group of tumors is characteristic of Phase I preliminary trials but makes it quite difficult to compare results with series published in the literature. The pyriform sinus was massively involved with tumor in 9 of the patients treated with neutrons alone and in 3 of the patients treated with mixed beam regime. Also, one patient in the mixed beam group had a T2 second primary in the oral tongue at the time of presentation. The neutrons only and mixed beam groups are not strictly comparable and will be considered separately in the remainder of the report.

Supraglottic laryngeal tumors: The usual convention of dividing the larynx into 3 regions was followed: (1) the supraglottis which consists of the epiglottis, the aryepiglottic folds, the arytenoids, and the ventricular bands with its inferior margin

being a horizontal plane passing through the ventricular apex (2) the glottis consisting of the true vocal cords (including the anterior and posterior commissures) with the inferior margin being a horizontal plane one cm below the ventricular apex and (3) the subglottis which is the region extending from the lower glottic margin to the lower border of the cricoid cartilage. A total of 11 patients with advanced laryngeal tumors were treated with fast neutrons during the period from March 1975 through December 1976. Of these 2 patients received only preoperative neutron beam irradiation, one patient received only a neutron boost of 500 cGy_n after having received 5040 cGy, conventional photon irradiation and one patient declined further neutron treatments after having received only 120 cGy_n in 2 fractions. The patient receiving the neutron boost had no local control of tumor and died 3 months after completing treatment. The patient who received only 2 neutron treatments was given an additional 5895 cGy, with megavoltage photons at another institution and apparently was disease free 3 months after completing therapy at which time he was lost to follow up. One of the preoperative patients received 1400 cGy_n to the primary and upper neck and 4500 cGy, photon radiation to the lower neck and supraclavicular region. He had severe wound healing problems and died of a carotid artery rupture. The other preoperative patient received 1475 cGy_n to the primary and upper neck and 4950 cGy, photon irradiation to the lower neck and supraclavicular area. At the time of surgery he was found to be disease free at the primary site but had a residual tumor in the cervical nodes (original staging T3N2). He developed a pharyngeal-cutaneous fistula after surgery and had some aspiration problems. He was lost to follow up 15 months after surgery and at that time had no tumor in the neck but did have widespread metastatic disease. Two other patients received only neutron radiation to areas of cervical adenopathy at times of 5 and 12 months respectively after having had definitive local photon radiation therapy and a surgical resection of the laryngeal primary. This leaves 6 patients with advanced laryngeal tumor treated for cure with neutron or mixed beam teletherapy who constitute the material for the present report.

Of these 6 patients 5 were treated with neutrons alone and one was treated with the mixed beam regime. The mean age of these patients was 60.5 years (range 35-86) with the male:female ratio being

Table 1

T and N staging system for hypopharynx

T-staging	
T1	Tumor confined to site of origin
T2	Tumor extends to adjacent site or region without fix of hemilarynx
T3	Tumor extends to adjacent site or region with fix of hemilarynx
T4	Massive tumor invading bone or soft tissues of neck
N staging (also applicable for larynx)	
N0	Clinically negative nodes
N1	Single homolateral node <3 cm in greatest dimension
N2	Single homolateral node 3-6 cm in greatest diameter or multiple homolateral nodes <6 cm in greatest diameter
N3	Massive homolateral nodes >6 cm in greatest diameter or bilateral or contralateral nodes

Table 2

Number of patients with hypopharyngeal tumors having a stage less than

	Neutrons only (10 patients)					Mixed beam (6 patients)				
	T1	T2	T3	T4	Total	T1	T2	T3	T4	Total
N0	0	0	0	0	0	0	0	0	0	0
N1	0	0	2	0	2	0	0	0	0	0
N2	0	1	1	0	2	0	0	1	0	1
N3	0	2	2	2	6	0	3	2	0	5
Total	0	3	5	2	10	0	3	3	0	6

Table 3

T staging system for supraglottic larynx

T1	Tumor confined to site of origin with normal cord mobility
T2	Tumor involves adjacent supraglottic sites or glottis without cord fixation
T3	Tumor limited to larynx with cord fixation or extension to hypopharynx or pre-epiglottic space or both
T4	Massive tumor extending beyond larynx to involve oropharynx, soft tissues of neck or thyroid cartilage

4:2. Almost every case had a significant history of ethanol and tobacco abuse. All patients underwent direct endoscopic examination under anesthesia. One patient had biopsy proven squamous cell carcinoma. One patient treated with the mixed beam regime had a poorly differentiated lesion while in the gr

Table 4

Number of patients with supraglottic laryngeal tumors having a given stage lesion. Neutrons only and mixed beam (6 patients)

	T1	T2	T3	T4	Total
N0	0	0	0	0	0
N1	0	0	0	0	0
N2	0	0	0	1	1
N3	0	1	1	3	5
Total	0	1	1	4	6

Table 5

Comparison between the present results and the results of conventional radiation therapy for advanced lesions of the hypopharynx and supraglottic larynx

	Fraction disease free
Hypopharynx	
Present series (flat portion of curve)	
Neutrons only	0.4
Mixed beam	0.4
GLE & SHIGEMATSU (1976)	
Radiation alone 3 year NED)	
Lymph sinus (T3N0-3)	0.04
Postcricoid (T3N0-3)	0
Posterior pharyngeal wall (T3N0-3)	0.3
ARKS et coll (1978)	
Radiation and surgery 2 year NED)	
Lymph sinus (T3)	0.4
(T4)	0.37
Supraglottic larynx	
Present series (flat portion of curve)	0.17
ROSSIN et coll (1974)	
3 year NED)	
T3	0.45
T4	0.49
ANG (1973) (3 year NED)	
T3	0.71
T4	0.23

treated with neutrons alone 2 lesions were poorly differentiated and 3 lesions were well differentiated. The primary lesions were staged according to the recommendations of the American Joint Committee for Cancer Staging and End Result Reporting which is shown in abbreviated form in Table 3. The nodal

status was assessed using the same scheme as for the hypopharyngeal lesions as indicated in Table 1. The distribution of laryngeal tumors according to this staging system is shown in Table 4. As in the case of the hypopharynx it is important to note the advanced disease status of the patients. This often made it difficult to determine precisely where in the larynx the tumor arose but based upon the joint clinical evaluation of the examining physicians (including the otolaryngologist who performed the endoscopic examination) all 6 lesions appeared to originate in the supraglottic region.

Results

Hypopharyngeal tumors All patients who have not either died or exhibited a local recurrence at the primary site have been followed up for a minimum of 26 months. Because of the difficulty in distinguishing between residual tumor and radiation induced ulceration a patient was not considered as having achieved a local control until either no possibly malignant areas were noted on examination or until biopsies of any such areas were proved to be negative. If during follow up a possibly malignant area showed itself to be residual tumor then the patient was categorized as not having achieved an initial local control. In a few cases the patient died before he had been adequately evaluated and if no autopsy was obtained such patients were assumed to be treatment failures. All follow up times are measured from the date radiation therapy was completed.

In the group of 10 patients with hypopharyngeal tumor treated with neutrons alone one was given 150 cGy_{av} on a Monday-Friday basis 4 were given 100 cGy_{av} on a Monday-Wednesday-Friday basis and 4 were given 75 cGy, on a Monday-Tuesday-Thursday-Friday basis. One patient was treated in still another way and received 100 cGy, on a Monday-Tuesday-Thursday-Friday basis. Because of holiday scheduling there were of course some modifications to these regimes. In this group of patients as well as other groups with tumors of different head and neck sites (LARAMORE et coll 1979 1980) no obvious differences were found in patient tolerance to the treatment among the various neutron-only treatment regimes. All patients except one completed their planned course of treatment and received between 1900 and 2200 cGy, to the primary tumor volume and any area of massive cervical adenopathy. The other patient received 1600

cGy_m, at which time a second primary was noted in a region of the cervical esophagus at the lower border of the neutron field. The primary had totally regressed as had the neck node (originally staged T3N1) and so a ⁶⁰Co photon field was added to the lower neck. An additional 2000 cGy_m was given to the primary and 5400 cGy_m to the esophageal lesion using right and left posterior oblique wedge fields. This patient had complete regression of both primary lesions. She died 2 months later of a pulmonary infection and at autopsy she was disease free at both sites and furthermore no evidence of distant metastases was found. Counting this patient the initial complete remission rate was 4/10 (40%). The subset with complete remission included 2 patients with T2 lesions and 2 patients with T3 lesions. All 4 patients died free of disease at the primary sites at respective times of 2, 5, 6 and 10 months. The patient who died at 6 months did so because of airway obstruction which was considered to be a treatment complication. The patient who died at 11 months did so because of pulmonary metastases although he did have some problems with laryngeal edema 8 months after completing therapy. The survival of the subset of patients who did not achieve an initial local control ranged between 2.5 and 11 months (mean 6.25 months). Of these 2 patients had an attempted surgical resection of their residual disease and lived 10 and 14 months respectively after the resections. One patient was free of disease at autopsy while one had residual tumor in the neck. A graphical display of the actuarially calculated (American Joint Committee for Cancer Staging and End Result Reporting 1977) fraction of patients disease free at the primary site is shown as the lower curve in Fig. 1. The actuarial method was used to maximize the information obtained from a group of patients with a spectrum of follow up times.

In the group of patients treated with the mixed beam regime the total radiation doses to the primary site ranged between 840 and 900 cGy_m + 3600 to 4140 cGy. In terms of an approximate photon dose (assuming RBE = 3) the doses ranged between 6300 and 6660 cGy equivalent. The initial complete remission rate for the mixed beam group of patients was 5/6 (83%). Of these 2 recurred within 2 months bringing the percentage with any significant duration of local control to about that of the group treated with neutrons alone. A graphical display of the actuarially calculated (American Joint Committee for Cancer Staging and End Result Re-

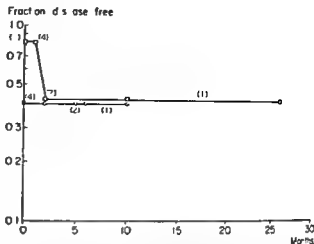


Fig. 1. Fraction of patients with hypopharyngeal tumor disease free at the primary site as a function of time after completing radiation therapy. The curves were calculated using the actuarial method (American Joint Committee for Cancer Staging and End Result Reporting). Ten patients treated with neutrons alone (□) 6 patients treated with a mixed beam regime (■). The ordinate is shown on a logarithmic scale and the numbers in parentheses indicate the number of patients at risk for the given data points.

porting) fraction of patients free of disease at the primary site is shown in the upper curve in Fig. 1. The one patient (stage T3N3) who did not achieve an initial complete remission died 2 months after completing therapy when advancing tumor eroded through a carotid artery. Only one patient in the subgroup who attained an initial complete remission is still alive and although he continues to be free of local and regional disease at 26 months he had developed pulmonary metastases at 22 months. One other patient developed pulmonary metastases at one month and another developed hepatic metastases at 10 months. A surgical salvage was attempted in one patient who recurred at 2 months but it was only partially successful. Although no untoward postoperative complications occurred the patient died of progressing tumor 3 months later. Excluding the one patient who is still alive the other 4 patients who achieved an initial complete remission had a mean survival of 4.5 months (range 1-10).

Both groups of patients developed the expected treatment reactions of skin erythema, mucositis, sore throat, dysphagia, decreased salivary function and altered taste sensation. In the mixed beam group of patients these sequelae were about the same as would be expected from conventional photon irradiation to comparable dose levels. No patient treated with the mixed beam regime required a break in treatment. The treatment reactions were more severe in the group of patients treated with neutrons

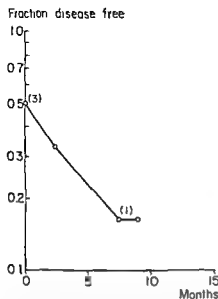


Fig 1. Fraction of patients with tumor of the supraglottic larynx disease free at the primary site as a function of time after completing radiation therapy. The curve was calculated using the actuarial method (American Joint Committee for Cancer Staging and End Result Reporting) and includes patients treated both with neutrons alone and with the mixed beam regime. The ordinate is shown on a logarithmic scale and the numbers in parentheses indicate the number of patients at risk for the given data points.

alone. Three patients required a 2 week break because of a severe moist skin desquamation and 2 patients developed significant laryngeal edema at 6 and 8 months after completing therapy. All patients had an acceptable vocal quality at the time of completing radiation therapy.

Supraglottic laryngeal tumors. All patients have been followed up until they have either died or exhibited a local recurrence. The criteria for assuming that a patient achieved an initial local control of his tumor are the same as for the group with hypopharyngeal tumors. Again, all follow up times are measured from the completion of radiation therapy.

A total of 6 patients with advanced tumors of the supraglottic larynx were treated for cure. 5 patients were treated with neutrons alone and one patient was treated with the mixed beam regime. In the patients treated with neutrons alone, one received 150 cGy_n on a Monday-Wednesday-Friday basis, 2 received 100 cGy_n on a Monday-Wednesday-Friday basis and 2 received 75 cGy_n on a Monday-Tuesday-Thursday-Friday basis. Total doses to the primary site and any areas of massive cervical adenopathy ranged between 1900 and 2000 cGy_n. The patient who was treated using the mixed beam regime received 900 cGy_n + 3960 cGy_x = 6660

cGy equivalent. In 3 of these 6 patients (including the mixed beam patient) an initial complete remission was achieved but the average duration of this was only 6.3 months (range 2-9). The subset with complete remission included one patient with a T2 lesion, one patient with a T3 lesion, and one patient with a T4 lesion. In this subset, one patient died free of disease at 9 months and one patient exhibited a local recurrence at 2.5 months and underwent a surgical salvage procedure. This patient had received 1900 cGy_n to the primary and involved cervical neck nodes (initial stage T3N3) and severe postoperative wound healing problems occurred. Although this patient lived an additional 11 months after the initial surgery, repeated surgical manipulations were required to remove nonviable tissue and death was ultimately due to erosion through a carotid artery. This is in contrast with the patient receiving the mixed beam irradiation (initial stage T2N3) who underwent a salvage surgical procedure when he recurred at 7.5 months without showing any postoperative wound healing complications. This latter patient is still alive and doing well 42 months after the surgery. The 3 patients who did not achieve an initial complete remission had a mean survival of only 3.3 months (range 2-6). A graphical display of the actuarially calculated (American Joint Committee for Cancer Staging and End Result Reporting) fraction of patients disease free at the primary site appears in Fig 2.

The complications of radiation therapy were essentially the same as listed for the group of patients with hypopharyngeal tumors. In the group of patients treated with neutrons alone, one developed a marked subcutaneous edema shortly after completing treatment and another patient developed a moist desquamation severe enough to warrant a one week break in the treatment. The patient treated with the mixed beam regime had no untoward difficulty. All patients had an acceptable vocal quality after completing radiation therapy.

Discussion

The results of a Phase I preliminary trial carried out at this University were described in which fast neutrons from a cyclotron designed for physics research were used to treat patients with advanced tumors of the hypopharynx and supraglottic larynx. Because this was a Phase I trial designed as much to investigate the toxicity of neutron radiation as to

gain clinical information about its tumoricidal potential: only patients with advanced lesions who were considered to have less than a 10 per cent 5 yr survival with conventional treatment modalities were accepted. The short survival times of the patients who did not show an initial complete response to therapy bear this out. In the case of patients with hypopharyngeal tumor it was possible to discuss separately the subgroups treated with neutrons alone and with a mixed beam regime. Although the numbers in the 2 groups are small, the results support other reports (GRIFFIN et coll. 1978, 1979; LARAMORE et coll. 1979, 1980) which indicate that the mixed beam regime is at least as effective as neutrons alone and that the side effects, both alone and in connection with subsequent surgery (GRIFFIN et coll. 1979), are no more severe than those associated with photon radiation alone. This is important since it provides a way of maximally utilizing treatment time on neutron therapy machines and this is likely to be fairly limited in the foreseeable future. The side effects of the neutron-only treatment regimes were more severe than the mixed beam regime and a high complication rate occurred in patients when a surgical salvage was attempted after full dose radiation. However, even when conventional photon radiation is used in a preoperative manner, VANDEN BROUCK et coll. (1977) found a high percentage of postsurgical complications for hypopharyngeal tumors.

In the group of patients with hypopharyngeal tumor, the local control rate as shown in Fig. 1 plateaued at about 0.4 for both the neutron and the mixed beam group. However, only one patient was at risk beyond 10 months. Four of the 9 patients with initial local control developed distant metastases, which accounts in part for the poor survival in spite of there being initial local control. The tendency of advanced lesions involving the pyriform sinuses to develop distant metastases has been previously documented (MARKS et coll.) and the present results tend to bear this out. This is particularly the case when the patients have advanced cervical adenopathy as well. A crude comparison between the plateau phase figures and the results quoted in the literature for the local control rate of advanced hypopharyngeal tumor is done in Table 5. INOUE & SHIGEMATSU break their results down according to site of origin and find considerably better results for posterior pharyngeal wall tumors than for tumors arising in either the pyriform sinus or the post-

cricoid regions. These figures come from their Table 2 excluding the long term survivors due to salvage surgery. MARKS et coll. quote figures for planned preoperative radiation followed by surgery at ~0.2 for T4 lesions of the pyriform sinus. Although the follow-up time of 2 years is considerably longer than the present ones, their figures are comparable with the plateau phase figures obtained with radiation alone.

A similar comparison has been made in Table 6 for the group of patients with supraglottic tumors. At 2 years, GHOSSEIN et coll. (1974) reported NE survival of 0.45 and 0.49 for T3 and T4 lesions, respectively. WANG reported corresponding results at 3 years of 0.21 for T3 lesions and 0.23 for T4 lesions, although it should be noted that 49 per cent of his patients were initially staged N0 and all of the present patients were stage N2-N3.

The advanced group of tumors in the present series makes comparison with the literature results quite difficult. The current investigation on the use of high LET neutron radiation for squamous cell carcinoma of the head and neck area is a randomized prospective trial which directly compares this with conventional photon radiation. Because of the morbidity of definitive neutron radiation alone, shown in the early Phase I trial, this institution and most of the other neutron institutions in the United States are using only a mixed beam treatment regime for the high LET arm. Preliminary results indicate that this is more effective at local control than the photon arm, but it is much too early to say anything about improved survival.

Conclusion

The analysis of a Phase I clinical trial testing the efficacy of fast neutron radiation therapy for advanced carcinoma of the hypopharynx and supraglottic larynx reveals local control rates comparable with photon radiation therapy of lesions which were probably less advanced. The control rate using a mixed beam (neutron/photon) treatment regime seems to be as good as that achieved with neutron radiation alone but with less associated adverse effects.

SUMMARY

The results of a Phase I clinical trial using fast neutron teletherapy for advanced squamous cell carcinoma of the hypopharynx and supraglottic larynx are reported. Ten patients with hypopharyngeal tumors were treated for cure using neutrons alone and 6 patients with hypophar-

real tumors were treated for cure using a combination of neutrons and photons as part of a mixed beam fractionation scheme. In the neutron only group the initial complete remission rate was 4/10 (40%). The mean survival of this group of patients with initial complete remission was only 5.75 months (range 2-10) but all patients without evidence of recurrent tumor at the primary site. In the mixed beam group the initial complete remission rate was 5/6 (83%) but the local control rate dropped to 41 per cent when displayed on an actuarial plot. Patients with advanced tumors of the supraglottic larynx were treated for cure: 5 with neutrons alone and 5 with a mixed beam regime. The initial complete remission rate was 3/6 (50%) but 2 of these patients rapidly died, which reduced the local control rate on an actuarial plot to 16 per cent. The relative adverse effects of neutron-only treatment regime appear to be substantially greater than those of the mixed beam treatment regime.

REFERENCES

- American Joint Committee for Cancer Staging and End Result Reporting: Manual for Staging of Cancer p 15 (1977)
- ATCHER G. Textbook of radiotherapy. Second edition p 255. Lea and Febiger Philadelphia 1973
- MACI J, JACKSON K, THROWER P and FOX M. An estimate of the patient risk in cyclotron radiotherapy using mouse testes as a biological test system. *Int J Radiat Oncol Biol Phys* 79 (1975) 727
- CHRISTENSEN G, THROWER P and FOX M. Cyclotron fast neutron RBE for various normal tissue. *Radiology* 115 (1975) 459
- PARKER R, FOX M and THROWER P. The relative biological effectiveness of cyclotron fast neutrons for early and late damage to the small intestine of the mouse. *Europ J Cancer* 10 (1974) 99
- JESEIN N, BATAINI J, ENVUYER A, STACEY P and KRISHNASWAMY V. Local control and site of failure in radically irradiated supraglottic laryngeal cancer. *Radiology* 112 (1974) 187
- PERFECT H, JESSE R, FLETCHER G and HAMBERGER A. Optimal treatment for the technically resectable squamous cell carcinoma of the supraglottic larynx. *Laryngoscope* (St. Louis) 83 (1975) 14
- FEINER D, ELTRINGHAM J, GLATSTEIN E and BAGSHAW M. Carcinoma of the larynx. Results of therapy in 713 patients. *Amer J Roentgenol* 117 (1973) 553
- GRIFIN T, BLASKO J and LARAMORE G. Results of fast neutron pilot studies at the University of Washington. In: *High LET radiations in clinical radiotherapy* p 23. Edited by G W Barendsen, J J Broerse and K Breur. Pergamon, Oxford 1979
- WEISBERGER E, LARAMORE G and TONG D. Complications of combined surgery and neutron irradiation for patients with advanced carcinomas of the head and neck region. *Radiology* 132 (1979) 177
- LARAMORE G, PARKER R, GERDES A, HEBARD D, BLASKO J and GROUDINE M. An evaluation of fast neutron teletherapy of metastatic cervical adenopathy from squamous cell carcinomas of the head and neck region. *Cancer* 42 (1978) 2517
- HARWOOD A and TIERIE A. Radiotherapy of early glottic cancer. *Int J Radiat Oncol Biol Phys* 5 (1979) 477
- HAWKINS N, RIDER W and BRYCE D. Radiotherapy of early glottic cancer. *Int J Radiat Oncol Biol Phys* 5 (1979) 473
- INOUE T and SHIGEMATSU Y. Hypopharyngeal carcinoma. Long term survivors following radical radiation therapy. *Acta radiol Ther Phys Biol* 15 (1976) 201
- KARIMA S, SNOW G, HASMAN A, CHANG S, KEILHOLTZ A and HOEKSTRA F. Dose response in radiotherapy for glottic carcinoma. *Cancer* 41 (1978) 1728
- LARAMORE G, GRIFIN T, GERDES A and PARKER R. Fast neutron and mixed (neutron/photon) beam teletherapy for grades III and IV astrocytomas. *Cancer* 42 (1978) 96
- GRIFIN T, TONG D, GROUDINE M, BLASKO J, KURTZ J, RUSSELL A and PARKER R. Fast neutron teletherapy for advanced carcinomas of the oral cavity and soft palate. *Cancer* 46 (1980) 1903
- MARKS J, KURNIK H, POWERS W and OGLRA J. Carcinoma of the pyriform sinus. An analysis of treatment results and patterns of failure. *Cancer* 41 (1978) 1008
- NASS J, BRADY L, GLASSBURN J, PRASASUINICHAI S and SCHATANOFF D. Radiation therapy of glottic carcinoma. *Int J Radiat Oncol Biol Phys* 1 (1976) 867
- NELSON J, CARPENTER R and PARKER R. Response of mouse skin and the C3HBA mammary carcinoma of the C3H mouse to X rays and cyclotron neutrons. Effect of mixed neutron photon fractionation schemes. *Europ J Cancer* 11 (1975) 891
- RASEY J, CARPENTER R, NELSON N and PARKER R. Cure of EMT-6 tumors by X rays or neutrons. Effect of mixed fractionation schemes. *Radiology* 123 (1977) 207
- STRYKER J, CHUNG C, CLEMENT J, CONNER G, STRAUSS M, ABT A and VELKLEY D. Tumor sterilization following high-dose preoperative irradiation for advanced cancer of the larynx of pyriform sinus. *Radiology* 132 (1979) 171
- VANDENBROUCK C, SANCHEZ H, LEFUR R, RICHARD J and CACHIN Y. Results of a randomized clinical trial of preoperative irradiation vs postoperative in treatment of tumors of the hypopharynx. *Cancer* 39 (1977) 1445
- WANG C. Megavoltage radiation therapy for supraglottic carcinoma. *Radiology* 109 (1973) 183
- WEAVER K, BICHSEL H, EENMAA J and WOOTTON P. Measurement of photon dose fraction in a neutron radiotherapy beam. *Med Phys* 4 (1977) 379
- WITHERS H. Biological basis for high LET radiotherapy. *Radiology* 108 (1973) 131
- WOOTTON P, ALVAR K, BICHSEL H, EENMAA J, NELSON J, PARKER R, WEAVER K, WILLIAMS D and WICKOFF W. Fast neutron beam radiotherapy at the University of Washington. *J Canad Ass Radiol* 76 (1975) 44

HYPOTHYROIDISM FOLLOWING ^{131}I THERAPY FOR HYPERTHYROIDISM
IN RELATION TO IMMUNOLOGIC PARAMETERS

G LUNDELL and L E HOLM

Immunologic reactions are known to be of importance in the development of hypothyroidism after treatment for hyperthyroidism. Postoperative hypothyroidism is more frequent when the surgical specimens reveal marked lymphoid infiltration (WHITESSELL JR & BLACK 1949 HARGREAVES & ARNER 1968). A higher incidence of postoperative hypothyroidism was found in patients who before treatment had demonstrable antibodies to thyroid antigens (HJORT & MOGENSEN 1962). ^{131}I treatment for hyperthyroidism is followed by hypothyroidism in a considerable proportion of the cases (LARSSON 1955 WERNER et coll 1957 BELING & EINHORN 1961 BECKER et coll 1971). A correlation between thyroid autoantibodies after ^{131}I treatment and hypothyroidism developing early after the therapy has been found (BLAGO 1960 SKANSE & LARSSON 1961 EINHORN et coll 1965) as well as correlation between the presence of thyroid antibodies before ^{131}I therapy and the development of hypothyroidism following such therapy (LUNDELL & JONSSON 1973). Whether this correlation found by LUNDELL & JONSSON still existed after a longer follow up period of the same patient group has now been investigated.

Material and Methods

The original series (LUNDELL & JONSSON) comprised 188 patients (144 females 44 males) with confirmed hyperthyroidism and in whom sera for deter-

mination of antibodies to thyroid antigens had been collected before and after ^{131}I therapy during the period 1963 to 1969. A further 3 patients also treated at this hospital during the same period and whose sera had been collected in the same manner could be added to the group of 188 patients. The median age of the 191 patients was 55 years (range 23-83 years).

Fourteen per cent of the patients ($n=27$) had no clinically demonstrable goiter, 46 per cent ($n=88$) a diffuse goiter and 40 per cent ($n=76$) a nodular goiter. The goiters were assessed on the basis of palpation as being moderately or markedly enlarged. None of the patients had previously received a therapeutic dose of ^{131}I but 37 patients had been operated upon.

The principles of ^{131}I therapy have been reported elsewhere (LARSSON BELING & EINHORN). The amount of ^{131}I administered at each treatment varied between 74 MBq (2 mCi) for patients without demonstrable goiter and 1295 MBq (35 mCi) for patients with large nodular goiters and the dose was calculated without knowledge of the thyroid antibody titers. When the initial ^{131}I dose was insufficient to control the hyperthyroidism further doses were given usually at a 3 month interval. Sixty-one per cent of the patients received one dose, 25 per cent two doses, 8 per cent three doses and 3 per cent of the patients received between five and seven

doses. The mean initial dose administered was 270 MBq (7.3 mCi) and the mean total dose 470 MBq (12.7 mCi, range 74–3700 MBq).

The patients were examined regularly after treatment at intervals of 2 to 4 months during the first year and subsequently at longer intervals, usually 6 months to one year, until they developed hypothyroidism or up till 31 December 1978. For patients who received more than one ^{131}I treatment, the follow-up period always dated from the time of the initial treatment. Classified as hypothyroid were all patients requiring permanent thyroid replacement therapy. The diagnosis of hypothyroidism was based on the clinical condition of the patient supported by laboratory analyses of sera and radioiodine tracer tests. If permitted on clinical grounds, thyroid replacement therapy was generally avoided during the first 4 months after the ^{131}I therapy since transient hypothyroidism may occur within the first months of such therapy.

Sera were collected from each patient before therapy and at intervals thereafter during the first year afterwards. Antibodies to thyroid cytoplasmic antigen were determined by Coons' direct immunofluorescence technique (ROITT & DONIACH 1958; HOLBOROW et al. 1959). Antibodies to thyroglobulin were determined by the Boyden passive hemagglutination test with tanned red cells (DERRIEN et al. 1948; BOYDEN 1951; WITEBSKY & ROSE 1956). Patients with titers of less than 1/20 of antibodies to thyroglobulin were classified as negative and those with titers of 1/20 or more as positive.

The cumulative incidence of hypothyroidism was calculated using standard life table techniques. The statistical methods used were the log rank test and the Student's *t* test.

Results

The cumulative incidence of hypothyroidism after ^{131}I treatment for hyperthyroidism is shown in Fig. 1. After one year 25 per cent of the patients without demonstrable goiter, 13 per cent of those with diffuse goiter and 5 per cent of the patients with nodular goiter were hypothyroid. At the end of the follow-up, the cumulative incidence of hypothyroidism was 63 per cent for patients without goiter, 57 per cent for those with diffuse goiter and 36 per cent for patients with nodular goiter. The differences were statistically significant ($p < 0.01$) be-

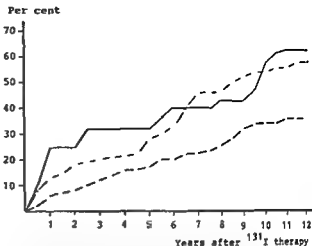


Fig. 1 Cumulative incidence of hypothyroidism after ^{131}I therapy for hyperthyroidism: — No demonstrable goiter ($n=77$); - - Diffuse goiter ($n=88$); ···· Nodular goiter ($n=76$).

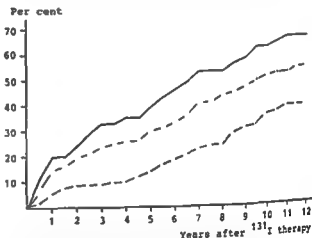
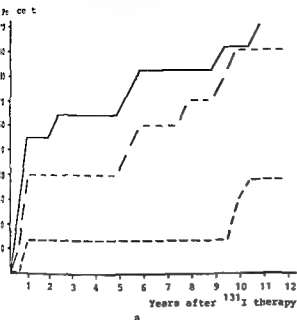


Fig. 2 Cumulative incidence of hypothyroidism after ^{131}I therapy in patients with or without thyroid antibodies before therapy. Patients with antibodies to both cytoplasmic antigen and thyroglobulin are included in both seropositive groups: — With antibodies to cytoplasmic antigen; - - With antibodies to thyroglobulin; ···· Without antibodies.

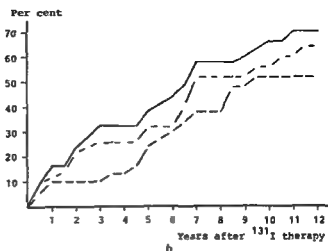
tween the group with nodular goiter and each of the two other groups, but not between the group with diffuse goiter and that without goiter.

The incidence of hypothyroidism in the 37 patients operated upon before ^{131}I treatment was similar to that of the other 154 patients.

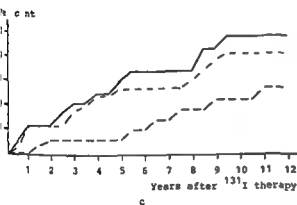
Of the 75 patients who before treatment had demonstrable antibodies to thyroid cytoplasmic antigen, 20 per cent were hypothyroid within one year and 66 per cent within 12 years of the treatment, as compared with somewhat lower figures for those with demonstrable antibodies to thyroglobulin ($n=88$, Fig. 2). Patients who lacked both antibodies ($n=81$) exhibited a lower cumulative incidence than



a



b



c

Fig 3 Cumulative incidence of hypothyroidism after ^{131}I therapy in patients with a) no goiter b) diffuse goiter c) nodular goiter. Patients with antibodies to both cytoplasmic antigen and thyroglobulin are included in both seropositive groups — With antibodies to cytoplasmic antigen ~ ~ Without antibodies

Patients with antibodies to cytoplasmic antigen or thyroglobulin or both ($p < 0.05$) 6 per cent within one year as compared with 39 per cent within 12 years of therapy.

Fig 3 demonstrates the significance of the presence of thyroid antibodies before treatment in the development of hypothyroidism in relation to the type of goiter. Six of the 11 patients without demonstrable goiter (Fig 3a) and who displayed antibodies to thyroid cytoplasmic antigen became hypothyroid within one year and all the cases were hypothyroid within 12 years of the treatment. Patients with demonstrable antibodies to thyroglobulin ($n=10$) had an incidence of hypothyroidism that was similar to that of patients with demonstrable antibodies to thyroid cytoplasmic antigen although somewhat delayed. In those patients without detectable antibodies before treatment ($n=14$) 14 per cent were hypothyroid within one year and 38

per cent within 12 years of treatment. The difference between the seropositive (cytoplasmic antigen or thyroglobulin or both) and seronegative groups was statistically significant ($p < 0.01$).

In the group of patients with diffuse goiter (Fig 3b) and presence of antibodies to thyroid cytoplasmic antigen before therapy 14 per cent of the cases were hypothyroid within one year and 63 per cent within 12 years. Patients who before treatment had antibodies to thyroglobulin had a similar but somewhat lower cumulative incidence of hypothyroidism. Thirty-three patients had no detectable antibodies before treatment and they had a slightly lower cumulative incidence than the seropositive patients. However the differences between the three curves were not statistically significant.

Seventy-six patients had a nodular goiter (Fig 3c) and the cumulative incidence of hypothyroidism was lower for patients without demonstrable antibodies

before therapy ($n=34$) but the differences were not statistically significant

Fig 4 shows the cumulative incidence of hypothyroidism in patients with demonstrable antibodies to thyroid cytoplasmic antigen or thyroglobulin or both before therapy in relation to the type of goiter. It seems evident that the presence of these antibodies sooner or later results in hypothyroidism in almost all patients without goiter. The group of diffuse goiter had a similar progressive hypothyroidism although at a slower rate. A longer follow up period is needed to ascertain whether the group with nodular goiter reaches a plateau as is suggested in the figure.

Of the 119 patients without demonstrable antibodies to thyroid cytoplasmic antigen before the ^{131}I therapy (Fig 5) 52 developed such antibodies afterwards while 67 remained seronegative with respect to cytoplasmic antigen titers for at least one year. A significantly higher incidence of hypothyroidism was found in the group becoming seropositive ($p<0.01$). Of 104 patients without demonstrable antibodies to thyroglobulin before therapy 33 developed such antibodies within one year. No correlation was found between the incidence of hypothyroidism and the presence of antibodies to thyroglobulin developing after the therapy in comparison with those remaining seronegative for that antibody. Patients with diffuse goiter or not palpable thyroid glands more often developed antibodies to thyroid cytoplasmic antigen following therapy than patients with nodular goiter.

Discussion

^{131}I therapy for hyperthyroidism is followed by hypothyroidism in a considerable number of patients. Between 7 and 20 per cent of the patients become hypothyroid during the first year after therapy and the cumulative incidence increases to 25 to 50 per cent within 7 to 12 years of treatment (BELING & EINHORN). At present all patients treated with ^{131}I for hyperthyroidism between 1951 and 1977 at Radiumhemmet are analysed. The results after the analysis of the first 3000 of these cases show that the cumulative incidence of hypothyroidism increases steadily to 70 per cent within 25 years after such therapy.

^{131}I therapy for hyperthyroidism may trigger an autoimmune reaction manifested as an increase in humoral thyroid antibodies (EINHORN et coll 1965

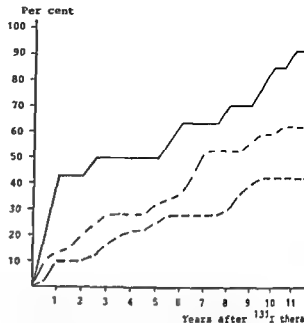


Fig 4 Cumulative incidence of hypothyroidism after ^{131}I therapy in patients with thyroid antibodies to cytoplasmic antigen, thyroglobulin or both before therapy — No goiter — Diffuse goiter — Nodular goiter

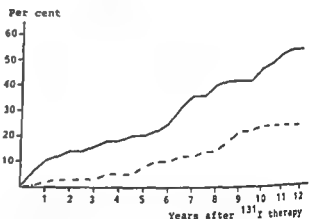


Fig 5 Cumulative incidence of hypothyroidism after ^{131}I therapy in patients without antibodies to cytoplasmic antigen before therapy — Seropositive to cytoplasmic antigen after therapy — Seronegative to cytoplasmic antigen after therapy

O GORMAN et coll 1964). This also occurred after ^{131}I therapy in euthyroid subjects (EINHORN et coll 1966). Previously it was shown that patients who had demonstrable antibodies before ^{131}I treatment developed hypothyroidism to a greater extent than those without such antibodies (LUNDELL & JONSSON). The present results show that this difference persists as long as 12 years after treatment. In the previous series hypothyroidism developing within one year after the ^{131}I therapy also occurred significantly more often in patients without demonstrable antibodies to thyroid cytoplasmic antigen before

tment but who developed such antibodies after treatment than in those who remained seronegative (LUNDELL & JONSSON). With the prolonged observation period of the present series a significant difference was found also for hypothyroidism developing more than one year after the therapy. The increase in thyroid antibodies after ^{131}I therapy is temporary and lasts about one year and at the time of hypothyroidism the level of humoral antibodies usually is at pre-treatment or lower levels (EINHORN et coll 1965, 1966). It is thus not likely that this humoral autoimmune reaction caused by ^{131}I therapy can alone account for the continued increase in the cumulative incidence of hypothyroidism many years after treatment. It is not known whether ^{131}I therapy also triggers an autoimmune mediated response. If so, such a response could contribute to the progressive development of hypothyroidism. Another explanation could be a progressive atrophy similar to that which has been observed after external irradiation (EINHORN et coll 1965). The present analysis shows that the results previously reported at an early stage of the same group of patients (LUNDELL & JONSSON) are valid also for a longer follow up period. It is evident that thyroid antibodies—and mainly those to thyroid cytoplasmic antigen—as well as the type of goiter are of importance in the development of hypothyroidism after ^{131}I therapy for hyperthyroidism. Immunologic factors must therefore be taken into account when comparing the results of different ^{131}I treatment schedules for hyperthyroidism. The level of thyroid antibodies before treatment could be a valuable factor when determining the dose of ^{131}I administered for the treatment of hyperthyroidism. Patients with a high risk of developing hypothyroidism could possibly be treated with lower doses, thereby curing them without unnecessary delay but also bringing about the onset of hypothyroidism more rapidly. Some of the problems arising in the follow up of these patients would then be diminished.

SUMMARY

A series of 191 patients with hyperthyroidism treated with ^{131}I was examined for the presence of antibodies to thyroid cytoplasmic antigen and to thyroglobulin before and after therapy and followed up for 12 years. Patients with thyroid antibodies before therapy had a significantly higher incidence of hypothyroidism than those without

demonstrable antibodies ($p < 0.01$). A higher incidence of hypothyroidism occurred in the presence of antibodies to thyroid cytoplasmic antigen than to thyroglobulin. Patients without demonstrable antibodies to thyroid cytoplasmic antigen before the therapy and who afterwards developed such antibodies had a significantly higher incidence of hypothyroidism than those who remained seronegative ($p < 0.01$).

ACKNOWLEDGEMENTS

The authors would like to thank Mrs Elisabeth Bjurstedt for her excellent technical assistance and Mr Bo Nilsson for the statistical calculations. This investigation was supported by grants from the Swedish Society of Medical Sciences (Project No. 185/78) and from the Cancer Society in Stockholm (Project No. 77/8).

REFERENCES

- BECKER D V, MCCONAHEY W M, DOBYS B M, TOMPAINS E, SHELIN G E and WORAMIAN J B. The results of radioiodine treatment of hyperthyroidism. A preliminary report of the Thyrotoxicosis Therapy Follow up Study. In: Further advances in thyroid research Vol 1 p 603. Edited by K. Fellinger and R. Hofer. Verlag der Wiener Medizinischen Akademie Vienna 1971.
- BELING U and EINHORN J. Incidence of hypothyroidism and recurrences following ^{131}I treatment of hyperthyroidism. *Acta radiol* 56 (1961) 275.
- BLAGG C R. Antibodies to thyroglobulin in patients with thyrotoxicosis treated with radioactive iodine. *Lancet* II (1960) 1364.
- BOYDEN S V. The absorption of proteins in erythrocytes treated with tannic acid and subsequent haemagglutination by antiprotein sera. *J exp Med* 93 (1951) 107.
- DEBRIEN Y, MICHEL R et ROCHE J. Recherches sur la preparation et les proprietes de la thyroglobuline pure. *I Biochim biophys Acta* 2 (1948) 454.
- EINHORN J, FAGRAEUS A and JONSSON J. Thyroid antibodies after ^{131}I treatment for hyperthyroidism. *J clin Endocr Metab* 25 (1965) 1218.
- — — Thyroid antibodies in euthyroid subjects after iodine ^{131}I therapy. *Radiat Res* 28 (1966) 296.
- IRVINE W J. Thyroid auto-immunity as a disorder of immunological tolerance. *Quart J exp Physiol* 49 (1964) 324.
- HARGREAVES A W and GARNER A. The significance of lymphocytic infiltration of the thyroid gland in thyrotoxicosis. *Brit J Surg* 55 (1968) 543.
- HIORT T and MOGENSEN E F. Thyroid autoantibodies. A study on the occurrence of circulating thyroglobulin antibody and complement fixing thyroid auto-antibody and an evaluation of the importance of these antibodies in the development of post-operative myxoedema. *Acta med scand* 171 (1962) 289.

- HOLBOROW E J, BROWN P C, ROITT I M and DONIACH D Cytoplasmic localization of complement fixing auto antigen in human thyroid epithelium *Brit J exp Path* 40 (1959) 583
- LARSSON L G Studies on radioiodine treatment of thyrotoxicosis with special reference to the behaviour of the radioiodine tracer tests *Acta radiol* (1955) Suppl No 126
- LUNDELL G and JONSSON J Thyroid antibodies and hypothyroidism in ^{131}I therapy for hyperthyroidism *Acta radiol Ther Phys Biol* 12 (1973) 443
- OGORMAN P, STAFFURTH J B and BALLENTYNE M R Antibody response to thyroid irradiation *J clin Endocr Metab* 24 (1964) 1072

- ROITT I M and DONIACH D Human auto immune thyroiditis. Serological studies *Lancet* II (1958) 1077
- SKANSE B and NILSSON S B Thyroid antibodies in hypothyroidism *Acta med scand* 170 (1961) 461
- WERNER S C, COELHO B and QUIMBY E H Ten year result of ^{131}I therapy of hyperthyroidism *Bull NY Acad Med* 33 (1957) 783
- WHITESSELL JR F B and BLACK H M A statistical study of the clinical significance of lymphocytic and fibrocytic replacements in the hyperplastic thyroid gland *J clin Endocr Metab* 9 (1949) 1202
- WITEBSKY E and ROSE N R Studies on organ specificity IV Production of rabbit thyroid antibodies in the rabbit *J Immunol* 76 (1956) 408

THYROID TREATMENT AND ITS POSSIBLE INFLUENCE ON OCCURRENCE OF MALIGNANT TUMORS AFTER DIAGNOSTIC ^{131}I

L E HOLM

Previously HOLM et coll (1980 a b) found that the number of malignant thyroid tumors in a population of 10 133 patients who had received diagnostic doses of ^{131}I for possible malignant thyroid tumor or for thyroid dysfunction did not differ from the number expected from the Swedish Cancer Registry data. When deaths were accounted for the mean follow up period was 17 years. Nine of these patients were reported to the Registry as having a malignant thyroid tumor against an expected figure of 8.3. On the other hand a large difference was found between the number observed and the number expected on the basis of the risk estimates for radiation induced malignant thyroid tumors given by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 1977) when these risk estimates were used after correction for the actual size of the thyroid glands (the computed number of such tumors was 47-124 (HOLM et coll 1980 a)). The observed incidence of malignant thyroid tumors could have been affected by thyroid surgery, thyroid hormone medication or other forms of thyroid treatment and the purpose of the present report is to present the extent to which such therapy was given after the administration of the diagnostic doses of ^{131}I .

Material and Methods

Between 1952 and 1965 diagnostic doses of on average 2.22 MBq (60 μCi)—0.11 to 34.8 MBq (3-940 μCi)—were given at Radiumhemmet to 8 047

female and 2 086 male patients for possible malignant thyroid tumor or thyroid dysfunction. The Swedish Cancer Registry records for 1958-77 were searched for the occurrence of malignant thyroid tumors in any of the 10 133 patients. In this follow up a latency period of 5 years was applied thereby excluding the first 5 person years for each patient and also tumors appearing in the Registry within 5 years of the first ^{131}I examination (HOLM et coll 1980 b).

In a sample of this population comprising all those patients born on days 5, 15 and 25 of each month the extent was analysed to which thyroid therapy was given during the follow up time which was taken as the period from the first administration of ^{131}I to 31 December 1977 or until death. The statistical sample numbered 968 patients (770 females and 198 males). This group did not differ from the whole patient population as regards either the mean age (44 years) or the mean activity of ^{131}I measured in the thyroid gland 24 hours after the administration of the nuclide (0.89 MBq).

The addresses of those patients still alive in August 1979 were obtained from the parish records. Patients who had died were divided into two groups according to whether they had survived for up to 5 years or for a longer period after the ^{131}I examination.

Since the latency period of 5 years had been applied in the previous calculations (HOLM et coll

1980b) the 97 patients dying within 5 years were not included in the present series. Of the remaining 871 patients of the statistical sample 187 had died by August 1979, 9 had emigrated and 10 could not be traced. The patients who had emigrated or who could not be traced were assumed to be alive on August 1979 and the thyroid treatment was classed as not known.

A questionnaire concerning any therapy for thyroid diseases was mailed to all 665 patients who were still alive in August 1979 and who could be located. It was accompanied by a letter explaining that an investigation was being carried out to ascertain the reliability of the diagnostic ^{131}I examination and its usefulness in medical practice. The patients were told that their answers would be helpful in deciding whether and if so how this examination could be improved. (The information so gathered will be analysed in a coming report.) No mention was made of the previous report on the incidence of malignant thyroid tumors following diagnostic doses of ^{131}I .

The patients were asked whether they had ever had thyroid surgery or received radioiodine therapy for any reason. In cases where such treatment had been given the name of the hospital and the approximate year of the treatment were requested. Patients that had received thyroid hormones or antithyroid drug therapy were requested to give the year when it was introduced. Those that had taken thyroid hormones as the sole form of treatment ($n=102$) were asked by telephone for how many years they had continued the medication. For 7 of these patients that could not be reached it was assumed that the drug had been taken continuously until the end of the follow up period on 31 December 1977.

Of the 665 patients receiving the questionnaire 628 answered and a further 22 could be reached by telephone. In 12 of the remaining 15 patients the records at the referring hospital yielded the required information. Thus in only 3 of the 665 patients were such data not obtained.

For the 187 patients who survived more than 5 years but had died by August 1979 every effort was made to gather information as to thyroid surgery, thyroid hormone medication or other forms of thyroid treatment received after the ^{131}I examination by searching their records at the referring hospitals at the hospitals where they had died or at any other relevant hospital mentioned in the records. In 133 of

these 187 patients the information was deemed adequate and reliable. In the remaining 54 patients the thyroid treatment was classed as not known.

Information on any form of thyroid treatment received after the ^{131}I examination was thus obtained for 795 of the 871 patients (91%) surviving more than 5 years.

The types of thyroid operation were classified as hemithyroidectomy or partial resection of one thyroid lobe, bilateral subtotal thyroidectomy and none known.

The data were recorded on a PDP 11/35 computer using an interactive input program. The analysis programs were run on the same computer.

Results

Of the 871 patients of the statistical sample who were still living 5 years after the first ^{131}I examination 507 (58%) received no form of thyroid treatment during the follow up period. Inadequate information was more common among the patients that died than among the survivors (Table 1).

Thyroid treatment of some kind was received within one year of the examination by one quarter of the 871 patients and some time during the follow up period by one third (Table 2). Surgery accounted for 69 per cent of the treatments given within the first year and for 58 per cent over the whole period. The most common surgical procedure was bilateral subtotal thyroidectomy (77%) followed by partial and hemithyroidectomy (10%), the remaining 13 per cent were classed as not known. No total thyroidectomy was performed on any patient. About one third of the patients undergoing surgery did not receive thyroid hormone therapy post operatively.

The second most common form of treatment was thyroid hormone medication that accounted for 27 per cent of the treatments given within the first year and for 35 per cent over the whole period. For the 102 patients given this as the sole form of treatment the mean period of medication was 10 years which was about two thirds of the follow up period for these patients. In 76 patients no information was available.

The distribution of the patients receiving thyroid surgery or hormone treatment according to the reason for performing the ^{131}I examination appears in Table 3.

Table 1

Distribution (per cent) of the statistical sample

Treatment	Patients		
	Alive (n=684)	Dead (n=187)	Total (n=871)
No thyroid treatment	62	44	58
Thyroid treatment	35	27	33
Not known	3	29	9

Table 2

Patients of the statistical sample surviving more than 5 years after the ¹³¹I examination distributed by the type of treatment

Type of treatment	Treatment given within 1 year of ¹³¹ I examination		Treatment given during follow up ending 31 December 1977	
	No	Per cent	No	Per cent
Surgery with or without thyroid hormone supplement therapy	147	17.0	168	19.4
Thyroid hormones only	57	6.5	107	11.7
Thyroid hormones + antithyroid drugs	3	0.3	8	0.9
Antithyroid drugs	6	0.7	7	0.8
¹³¹ I therapy	0	0	3	0.3
No treatment	582	66.8	507	58.2
Not known	76	8.7	76	8.7

Table 3

Patients undergoing thyroid surgery or given thyroid hormone treatment distributed by reasons for performing the ¹³¹I examination

Reason	Total No of patients	Surgery (per cent)	Thyroid hormone (per cent)
Possible malignant thyroid tumor	351	33	12
Hyperthyroidism	390	12	5
Hypothyroidism	152	1	70
Other reasons	75	4	13

Discussion

The extent to which thyroid surgery and thyroid hormone medication may have affected the incidence of malignant thyroid tumors in the present

patient population is difficult to establish. Surgery might be considered to reduce the probability of a tumor developing because of the removal of thyroid tissue. But it might also be argued that where surgery is not followed by thyroid hormone therapy the risk of a malignant tumor developing could be even greater than when no operation is performed because of the possible increase in the thyroid stimulating hormone level.

Thyroid hormones were administered to 12 per cent of the patients of the statistical sample. There is evidence that the growth of papillary and possibly follicular carcinomas can be arrested for some time by the administration of thyroid hormones (WERNER 1971). The risk of developing a radiation induced malignant tumor might have been reduced by the thyroid hormone therapy (DONIACH 1977). However, the efficacy of thyroid hormones in preventing thyroid tumor formation in humans many years after radiation exposure is still unknown.

Among the whole population of the 10 133 patients given diagnostic doses of ¹³¹I, 9 were reported to the Swedish Cancer Registry as having a malignant thyroid tumor. In 4 of these 9 patients thyroid surgery had been performed some time after the diagnostic examination and before the tumor diagnosis. 2 of them had received thyroid hormones postoperatively. In these 4 patients neither the thyroid surgery nor the administration of thyroid hormones was apparently effective in preventing development of the malignant tumor.

Three patients of the statistical sample had received radioiodine therapy for hyperthyroidism. The reason for this low number is that radioiodine therapy in this region of Sweden was at that time given almost exclusively at Radiumhemmet and all patients known to have received such therapy as well as external radiation therapy were excluded from the series (HOLM et coll 1980b). The intervals between the diagnostic and therapeutic ¹³¹I doses for the 3 patients of the sample were 11, 13 and 14 years respectively and in none of them was hyperthyroidism the reason for performing the initial diagnostic examination.

Radioiodine therapy seems not to be associated with an increased incidence of malignant thyroid tumors (HOLM et coll 1980c) but perhaps even reduces the probability of such tumors developing by virtue of the high doses of ¹³¹I administered (Committee on the Biological Effects of Ionizing Radiations 1972, UNSCEAR 1977).

Most of the 76 patients on whom no information concerning thyroid treatment could be obtained were among those that died. Because the information on these patients was sought only at hospitals, it is possible that this group includes patients who did not receive thyroid treatment and where adequate information could therefore not be obtained. Support for this possibility is found in the fact that the proportion of patients with no thyroid treatment was lower for the patients who died.

Because the determination of the effect of the various forms of thyroid treatment on the occurrence of radiation induced malignant thyroid tumors is unreliable, the fact that at least 58 per cent of the statistical sample did not receive any thyroid therapy during the time they were at risk is an important finding. In addition, among the 9 patients who were reported to the Swedish Cancer Registry because of a malignant thyroid tumor, 4 had had thyroid surgery and 2 had received thyroid hormones postoperatively. In view of the long follow up period, the difference between the number of malignant thyroid tumors observed in the 10 133 patients who had received diagnostic doses of ^{131}I and the number computed from UNSCEAR's risk figures is not ascribable to the thyroid treatment to more than possibly a small extent.

A conceivable explanation for this difference is the much lower dose rate of ^{131}I than of external irradiation, on which UNSCEAR's risk figures are mainly based. Furthermore, it is not known whether a radiation dose threshold for the induction of malignancy in humans exists, although the non-linear relationship between dose and effect implies that there is little if any risk at low dose levels in cases of cataract of the lens or impairment of fertility.

Animal experiments often indicate that the dose-response curve varies according to the types of Linear Energy Transfer (LET), the curve being sigmoid for low LET and linear for high LET. ISHIMARU et coll. (1971, 1979) found that the incidence of leukemia in humans after exposure to the radiation from atomic bombs was increased at doses of less than 1 Gy in Hiroshima but not in Nagasaki; the discrepancy might be due to differences in the types of radiation delivered—neutrons and gamma in Hiroshima and mainly gamma in Nagasaki.

The fact that also other authors have not disclosed any increased incidence of leukemia or malignant tumors at low doses of low LET radiation suggests

that the dose-response curve may not conform to the linear non threshold hypothesis (RALLISON et coll. 1974, FRIGERIO & STOWE 1976, SAKKA 1978).

In this series of 10 133 selected patients exposed to radiation from ^{131}I with a mean dose of 1.59 Gy for those less than 20 years of age and 0.58 Gy for those older than this at the time of the diagnostic examination (HOLM et coll. 1980a), no increased incidence of radiation induced malignant thyroid tumors was disclosed during the present follow up period. It would seem that the UNSCEAR risk figures for radiation induced malignant thyroid tumors are not applicable to the radiation emitted by ^{131}I . A longer follow up of this patient population is needed to elucidate if the results are confirmed over a longer observation period.

SUMMARY

The extent to which thyroid surgery, thyroid hormone medication or other forms of thyroid therapy were applied after diagnostic administration of ^{131}I has been determined in 871 patients of the 10 133 patients reported previously. The relevant information was obtained from questionnaires and from hospital records. Of the 871 patients, at least 58 per cent had received no form of thyroid treatment and 33 per cent had had some form of treatment, while in 9 per cent no information could be obtained. The large difference between the number of malignant tumors observed in the 10 133 patients after exposure to ^{131}I and the number expected from UNSCEAR's risk figures for radiation induced thyroid carcinoma (47–124 cases) cannot be ascribed to the various forms of thyroid treatment given after the ^{131}I administration.

ACKNOWLEDGEMENTS

The author would like to thank Mrs Elisabeth Bjurstedt and Mrs Karin Steen for their excellent technical assistance, Mr Ingemar Dahlqvist and Mr Anders Israelsson for programming and computing the data, Professor Gunnar Eklund for analysis of the epidemiologic problems and Mr Victor Braxton for the linguistic revision of the manuscript. This investigation was supported by grants from the National Institute of Radiation Protection, Stockholm, Sweden (Project SSI/P133/79).

REFERENCES

- Committee on the Biological Effects of Ionizing Radiation. The effects on populations of exposure to low levels of ionizing radiation. p. 122. Division of Medical Sciences, National Academy of Sciences, National Research Council, Washington D.C. 1972.
- DOBYSN B. M. Radiation hazard. Experience with therapeutic and diagnostic ^{131}I . In: Radiation as

- sociated thyroid carcinoma p 459 Edited by L J DeGroot L A Frohman E L Kaplan S Refetoff Grune & Stratton New York San Francisco London 1977
- DOMACH I Pathology of irradiation thyroid damage In Radiation associated thyroid carcinoma p 199 Edited by L J DeGroot L A Frohman E L Kaplan S Refetoff Grune & Stratton New York San Francisco London 1977
- FRIGERIO N A and STOWER S Carcinogenic and genetic hazard from background radiation In Biological and environmental effects of low level radiation p 385 Vol II Proceedings Series International Atomic Agency Vienna 1976
- HOLM L E EKLUND G and LUNDELL G (a) Incidence of malignant thyroid tumors in humans after exposure to diagnostic doses of iodine 131 II Estimation of thyroid gland size thyroid radiation dose and predicted versus observed number of malignant thyroid tumors J nat Cancer Inst 64 (1980) 1221
- LUNDELL G and WALINDER G (b) Incidence of malignant thyroid tumors in humans after exposure to diagnostic doses of iodine 131 I Retrospective cohort study J nat Cancer Inst 64 (1980) 1055
- DAHLQVIST I ISRAELSSON A and LUNDELL G (c) Malignant thyroid tumors after iodine 131 therapy A retrospective cohort study New Engl J Med 303 (1980) 188
- ISHIMARU T OTAKE M and ICHIMARU M Dose-response relationship of neutrons and γ rays to leukemia incidence among atomic bomb survivors in Hiroshima and Nagasaki by type of leukemia 1950-1971 Radiat Res 77 (1979) 377
- HOSHINO T ICHIMARU M OKADA H TOMIYASU T TSUCHIMOTO T and YAMAMOTO T Leukemia in atomic bomb survivors Hiroshima and Nagasaki 1 October 1950-30 September 1966 Radiat Res 45 (1971) 216
- RALLISON M L DOBYNS B M KEATING F R RALL J E and TYLER F H Thyroid disease in children A survey of subjects potentially exposed to fallout radiation Amer J Med 56 (1974) 457
- SAKKA M Background radiation dose and leukemia mortality in north Japan (In Japanese with English abstract) Nippon Acta radiol 38 (1978) 702
- United Nations Scientific Committee on the Effects of Atomic Radiation Sources and effects of ionizing radiation annex G p 361 United Nations New York 1977
- WERNER S C Medical (suppressive) versus surgical treatment In The thyroid A fundamental and clinical text Third edition p 475 Edited by S C Werner and S H Ingbar Harper & Row Publishers New York Evanston San Francisco London 1971

BONE ABNORMALITIES IN PATIENTS WITH MEDULLARY CARCINOMA OF THE THYROID

B. RASMUSSEN

Medullary carcinoma of the thyroid (MCT) constitutes about 10 per cent of all carcinomas of the thyroid. It differs from the other thyroid carcinomas in several ways, the most important being secretion of pathologically high amounts of the serum-calcium lowering hormone calcitonin, which probably is secreted during several years before the thyroid carcinoma becomes clinically recognizable as a palpable tumor in the thyroid.

MCT occurs in three apparently unrelated forms: either as a part of multiple endocrine neoplasia type 2a (MEN 2a) which incorporates MCT, parathyroid adenoma and pheochromocytoma, or multiple endocrine neoplasia type 2b (MEN 2b) which consists of MCT, pheochromocytoma, peripheral mucosal neuromas with or without marfanoid body build, and sporadic cases.

The present investigation was carried out in order to evaluate the frequency and appearance of bone abnormalities in patients with all three forms of MCT.

Material and Methods

All of 28 patients with microscopically proven medullary thyroid carcinoma, treated and controlled in this hospital in the period 1962 through 1978, were screened for bone abnormalities by clinical examinations and conventional radiography as well as by records also from other hospitals. Twenty-one patients had serum calcitonin measured. Additional examinations and measurements were performed in 5 patients: photon beam absorption of the radius and the heel, 25 hydroxy calciferol, immunoreactive parathyroid hormone and calcium in serum as well

as hydroxyproline in the urine. Immunoreactive calcitonin was measured by the method described by ALMQVIST *et al.* (1974). 25 hydroxy calciferol was measured according to the method described by LUND & SPØRSEN (1979). Parathyroid hormone (PTH) was measured by radioimmunoassay with a double antibody technique, the antibody being directed against the C-terminal end of the PTH molecule (RASMUSSEN *et al.* 1978).

The frequencies and appearances of bone affections were compared with those of 104 patients with other thyroid carcinomas, i.e. papillary, follicular and anaplastic carcinomas, treated in this hospital in the same period.

Results

Fourteen of the 28 patients with MCT had at least one bone abnormality (Table 1). All of the 6 patients who had the diagnosis of medullary thyroid carcinoma established before 32 years of age had bone abnormalities. Epiphyseal slip in the proximal femur occurred in 3 patients when they were 12 to 17 years old.

Schmorl nodes were present in 4 patients for more than 5 years without any herniation of the nucleus pulposus.

Bone cysts unaltered during at least 5 years appeared in 3 patients; the localizations were in the clavicle, the acetabulum, head of femur and the sacrum.

Table 1

Appearance of bone abnormalities in patients with medullary thyroid carcinoma

Age sex	Mar fanoid body build*	Dolicho- cephaly	Progna- thism	Ant chest de- formity	Scho- sis	Kypho- scolio- sis	Spina bifida *	Slipped epi- physis	Bone cysts	Schmorl nodes	Pes cavus	Re- marks
16 M	+	+	+	+	+		+	+		+	+	MEN 2b
17 M	+	+	+			+		+			+	MEN 2b
13 M	+	+	+		+		+				+	MEN 2b
30 F	+								+			
21 M										+		
31 M	+	+	+			+						MEN 2b
38 F	+				+			+	+	+	+	
59 F					+				+			Reckling hausen s disease
60 M					+							
59 F					+		+					
67 M			+							+		
43 M					+							
59 F					+							
67 M	+			+		+					+	

Further 3 patients had marfanoid body build but otherwise no bone abnormalities
Two S1 one C5-C6

Three patients had spina bifida (2 in S1 one in C5-C6) 2 of them had alpha₂ fetoprotein measured in serum it was normal in both

The mineral content of the extremities was measured in 5 patients with high serum calcitonin and was compared with that of 5 sex and age matched control patients with papillary or follicular thyroid carcinomas and normal serum calcitonin. The serum values of PTH, calcium and 25 hydroxy calciferol were within normal range in both groups except in one patient with MCT who had elevated 25 hydroxy calciferol because of D vitamin intake before the blood collection. The mineral content was slightly increased in one patient with MCT in the control group 3 of the patients had increased values.

Biopsy from the iliac crest was performed in 2 patients. In one an increased amount of osteoid was found in the other the biopsy was normal.

Four patients developed bone metastases: one patient osteolytic, another patient osteosclerotic. In the remaining 2 patients the type of metastases could not be diagnosed because they were found at a ¹³¹I scintigraphy and at autopsy respectively.

When compared with the bone abnormalities found in patients with other thyroid carcinomas, the frequency of scoliosis was equal (Table 2). In all 34 of 104 patients with other thyroid carcinomas

had bone abnormalities, practically all of them had scoliosis.

Three illustrative cases are described.

Case 1 A 16-year-old male was operated upon for a medullary thyroid carcinoma in 1962. Two years previously he was operated upon for a slipped femoral epiphysis and it was then noted that he had kyphoscoliosis, bilateral pes cavus and highly arched palate. In 1975 a second operation for recurrent MCT was performed. At this time it was noted that he had a marfanoid build, loose jointedness, hypertrophied gums with the teeth widely spaced as well as multiple mucosal neuromas located in the eyelids, the lips, the tongue and the larynx. Serum calcitonin was extremely high >30 000 pmol/l (normal range 120-290 pmol/l). PTH and serum calcium were normal and so was the urinary excretion of hydroxyproline. The mineral content was slightly increased to 41 and 32 units in the heel and the radius, normal values being 34±5 and 26±4 respectively. A biopsy from the iliac crest revealed increased amount of osteoid 83 per cent, normal spongiosa and normal surface resorption.

The combination of MCT, skin mucosal neuromas and marfanoid body build implies that this patient belongs to the group of MEN 2b; he had or has, however, no indication of pheochromocytoma.

Table 2

The types of bone abnormalities in 14 patients with medullary carcinoma of the thyroid (MCT) and in 34 patients with other thyroid carcinoma

Bone abnormality	MCT	Other thyroid carcinoma
Dolichocephaly	4	
Prognathism	5	
Funnel chest	1	
Carnate chest	1	
Scoliosis	8	30
Kyphoscoliosis	3	3
Spina bifida	3	1
Slipped femoral epiphysis	3	
Bone cysts	3	
Schmorl nodes	4	
Pes cavus	5	

Two S1 one C5-C6

Case 2 The diagnosis of MCT was made when the patient was 39 year old. At 14 years she was operated upon for left and right proximal femoral slipped epiphyses. A year previously she was operated upon for MCT. Clinical examinations and radiography revealed bilateral pes cavus, scoliosis, a few bone cysts scattered in both femora and acetalabulum as well as multiple Schmorl nodes. Three years later recurrence of the thyroid carcinoma developed and serum calcitonin rose to >140 000 pmol/l, PTH, calcium and 25 hydroxy calciferol in serum were normal and so was the bone mineral content. No mucosal neuromas and no signs of pheochromocytoma were found. The patient thus had MCT in its monosymptomatic form.

Case 3 As a 2 year-old boy this patient was operated upon for a megacolon and when he was 13 years old for a megalocystus. A subtotal thyroidectomy because of MCT was performed when he was 16 years old. The following year he was operated upon for a slipped femoral epiphysis on the left side and it was noted that he had bilateral pes cavus, scoliosis, pectus carinatum, spina bifida in S1 and Schmorl nodes in the lumbar vertebrae. As it further was found that he had a marfanoid body build, prognathism, dolichocephaly and multiple mucosal neuromas the patient was classified as having MEN 2b. During 1977 to 1978 serum calcitonin rose to 275 000 pmol/l, PTH and 25

hydroxy calciferol in serum, hydroxyproline excretion in the urine as well as the bone mineral content were all within normal ranges.

Discussion

In 1968 GORLIN et coll. noticed that in a few patients with MCT, pheochromocytoma and mucosal neuromas, marfanoid body build was an associated finding. In 1975 KHAIRI et coll. suggested the name MEN 3 for the combination of MCT, pheochromocytoma, mucosal neuromas with or without marfanoid body build, the latter being defined as a slender body build with a sparsity of body fat and poor development of the body musculature, occasionally accompanied by pes cavus, pectus excavatum and high arched palate.

CARNEY et coll. (1978) surveyed their 17 patients and an additional 73 patients from the literature, all having MEN 3 or as CARNEY et coll. call the syndrome—MEN 2b. They further included the following skeletal abnormalities: prognathism, elongation of the skull (dolichocephaly) and slipped femoral proximal epiphysis.

In the present series only 4 patients with MCT and various bone defects were found to have mucosal neuromas, i.e. they belong to the group of MEN 2b. All 4 patients had marfanoid body build and prognathism, but these were found in other patients with MCT as well.

The possible causes of these bone abnormalities could be (1) a disorder in connective tissue as in patients with Marfan syndrome, (2) a sustained effect of calcitonin on the bones or (3) a defect of a stem cell in the neural crest.

(1) *Marfan syndrome* is an autosomal dominant inheritable disease affecting the skeletal system, the aorta and the lens. MCKUSICK (1972) suggested that both the defects in the aorta and the lens as well as the bone disorders could be due to a defect in connective tissue, probably collagen. When compared with the bone defects in patients with the genuine Marfan syndrome, the following traits may occur also in patients with MCT: dolichocephaly, high arched palate, prognathism, anterior chest deformity, kyphoscoliosis, pes cavus, spina bifida and marfanoid body build (muscular underdevelopment and sparsity of body fat). In this comparison, bone cysts, Schmorl nodes and slipped femoral proximal epiphysis are limited to patients with MCT.

In none of the present patients could the main characteristics of the Marfan syndrome be found: ectopia lentis, dilation of the aorta and arachnodactyly. Hydroxyproline in the urine is occasionally increased in patients with Marfan syndrome (SJOERDSMA et coll 1958) however in 5 of the patients with various bone defects including 3 patients with MEN 2b it was normal.

MCT may appear as an autosomal dominant disease and in that case most often as a part of MEN 2a or to a lesser degree MEN 2b (CARNEY et coll). However most cases are non hereditary in the present series only 2 of 28 cases.

(2) *Sustained effect of calcitonin* Calcitonin is a polypeptide hormone normally produced and secreted by the c-cells in the thyroid gland. In cases of malignant development of the c-cells i.e. in medullary thyroid carcinoma calcitonin is secreted in abnormal high serum concentrations. The normal range of serum calcitonin is 120 to 290 pmol/l measured with the technique used in the present series. In patients with MCT serum calcitonin in the range of 6000 to 275000 pmol/l was measured.

The role of calcitonin in the normal human physiology is only partly known. It is generally accepted that the effect is the opposite of that of parathyroid hormone with the exception of the effect on phosphate, as both hormones improve the excretion of phosphate in the urine (ARDAILLOU et coll 1969, SINGER et coll 1969). SORENSEN et coll (1970) injected porcine calcitonin into both hypercalcemic and hypocalcemic patients and found a lowering of serum calcium in both groups. This hypocalcemic effect is probably due to an inhibition of bone resorption (BUVOET et coll 1970).

It has not been possible to recognize any effect of depletion of calcitonin i.e. in patients with the entire thyroid gland removed.

In hypersecretory states i.e. in patients with MCT a definite metabolic function of calcitonin has not been established. It may have been expected that serum calcium and serum phosphate were decreased in patients with high serum calcitonin but this has not been the case as reported by MELVIN et coll (1973) and RASMUSSEN (1978). The effect on bone tissue may be an increased mineral content in patients with longstanding high serum calcitonin. However this was not found by MELVIN et coll who assessed the bone mineral content in 18 patients with MCT nor in 5 of the present

patients who moreover had normal 25 hydroxycalciferol, serum calcium and PTH.

In 3 patients with MCT MELVIN et coll found that all parameters related to bone cell activity were markedly decreased. These findings were not confirmed in bone biopsies performed on 2 of the present patients.

It is possible that the absence of a demonstrable metabolic effect of the pathologically increased serum calcitonin in patients with MCT is due to the possibility that the encountered serum calcitonin is not the metabolically active form of calcitonin but for example is a nonactive precursor molecule of calcitonin (DOKK et coll 1976) or simply another calcitonin reacting immunologically like the normal calcitonin in the assay used in the present series but without the normal calcitonin effect on calcium, PTH and phosphate.

(3) *Stem cell in the neural crest* MCT develops from the c-cells in the thyroid gland. These cells have their origin in the neural crest (LE DOUARIN & LIÈVRE 1970) and so have cells in the pituitary, the pancreas, the stomach, the intestine and the adrenal medulla. The cells are named APUD-cells because they have common cytochemical properties (amine precursor uptake and decarboxylation). Tumors developed from the APUD-cells are called apudomas (PEARSE 1968). Apudomas share a common stem cell in the embryonal neural crest with neuromas, schwannomas and melanomas and these tumors together are called neurolophomas after a proposal from PEARSE & POLAK (1974).

A defect in this stem cell could apart from MCT lead to multiple neuromas which may be the cause of some of the bone abnormalities occurring in patients with MCT (spina bifida and scoliosis). Further HORST (1975) has suggested that neural crest cells migrate into and become a part of the musculoskeletal blastema and hence a possible cause for the various bone abnormalities present.

SUMMARY

The frequency and appearance of bone abnormalities in patients with medullary carcinoma of the thyroid were compared with those of patients with other thyroid carcinomas. Only in patients with medullary carcinoma the following abnormalities were found: dolichocephaly, prognathism, anterior chest deformity, bone cysts, Schmorl nodes, spina bifida, slipped femoral epiphysis, pes cavus and marfanoid body build. Not only patients with medullary carcinoma of the thyroid as part of

multiple endocrine neoplasia but also patients with otherwise unrelated medullary carcinoma had bone defects. The cause of the bone abnormalities is probably a defect of a stem cell in the embryonal neural crest rather than a sustained effect on bone tissue of the tumor produced calcitonin.

ACKNOWLEDGEMENT

Microscopic examination of the biopsy specimens was performed by Dr Inge Reimann, Orthopaedic Hospital Copenhagen.

REFERENCES

- ALMQUIST S, TELENUS-BERG M and WASTHED B. Serum calcitonin in medullary thyroid carcinoma. *Acta med scand* 196 (1974) 177.
- ADAILLOU R, FILLASTRE J P, MILHAUD G, ROUSSELET F, DELAUNAY F and RICHET G. Renal excretion of phosphate, calcium and sodium during and after a prolonged thyrocalcitonin infusion in man. *Proc Soc exp Biol Med* 131 (1969) 56.
- BAOET O L M, V D VEER SLUYS J, WILDIERS J and SMEENK D. Effects of longterm calcitonin administration to patients. In: *Calcitonin 1969*. Proceedings of the Second International Symposium p 531. Edited by S. Taylor and G. Foster. Heinemann, London 1970.
- CARNEY A J, SIZEMORE G W and HAYLES A B. Multiple endocrine neoplasia, type 2b. *Pathobiol Ann* 8 (1978) 105.
- V D DONK J A, V DAM R H, GOUDSWAARD J, HACKENG W H L and LIPS C J M. Precursor molecule for calcitonin. *Lancet* II (1976) 1133.
- LE DOUARIN N et LF LIEVRE C. Demonstration de l'origine neurale des cellules à calcitonine du corps ultimobranchial chez l'embryon de poulet. *C R Acad Sci Paris* 270 (1970) 2857.
- GORLIN R J, SEDANO H O, VICKERS R A and CERVENKA J. Multiple mucosal neuromas, pheochromocytoma and medullary carcinoma of the thyroid. A syndrome. *Cancer* 22 (1968) 293.
- HORST J H. Neural crest and early fore limb development in amphibia. *Anat Embryol* 147 (1975) 337.
- KHAIRI M R A, DEXTER R N, BURZYNSKI N J & JOHNSTON C C. Mucosal neuroma pheochromocytoma and medullary thyroid carcinoma. Multiple endocrine neoplasia type 3. *Medicine* 54 (1975) 89.
- LUND B and SØRENSEN O II. Measurement of 25-hydroxyvitamin D in serum and its relation to sun shine, age and vitamin D intake in the Danish population. *Scand J Clin Lab Invest* 39 (1979) 23.
- MCKUSICK V A. Heritable disorders of connective tissue. Fourth edition p 61. C V Mosby Company, Saint Louis 1972.
- MELVIN K E W, TASHJIAN A H and BORDIER P. The metabolic significance of calcitonin secreting thyroid carcinoma. In: *Clinical aspects of metabolic bone disease* p 193. Edited by B. Frame, A. M. Parfitt and A. H. Duncan. Excerpta Medica, Amsterdam 1973.
- PEARSE A G E. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C-cells and calcitonin. *Proc roy Soc B* 170 (1968) 71.
- and POLAK J M. Endocrine tumours of neural crest origin: neuroblastomas, pheochromocytomas and the APUD concept. *Med Biol* 52 (1974) 3.
- RASMUSSEN B. Magnesium and phosphate in the serum of patients with medullary carcinoma of the thyroid. *Clin chim Acta* 89 (1978) 279.
- ROESDAHL K R and LINDGREEN P. Parathyroid hormone and calcitonin in serum of patients with mammary carcinoma. *Acta radiol Oncology* 17 (1978) 269.
- SINGER F R, WOODHOUSE N J Y, PARKINSON D K and JOPLIN G F. Some acute effects of administered porcine calcitonin in man. *Clin Sci* 37 (1969) 181.
- SJOERDMA A, DAVIDSON J D, UDENFRIEND S and MITOMA C. Increased excretion of hydroxyproline in Marfan's syndrome. *Lancet* II (1958) 994.
- SØRENSEN O II, FRIIS TH, HINDBERG I and NIELSEN S P. The effect of calcitonin injected into hypercalcaemic and normocalcaemic patients. *Acta med scand* 187 (1970) 283.

FROM THE DEPARTMENT OF ONCOLOGY DIVISION OF GYNAECOLOGIC ONCOLOGY AKADEMISKA
Sjukhuset S 750 14 UPPSALA AND THE DEPARTMENT HISTOPATHOLOGY AND CYTOLOGY FALU
Sjukhus S 79191 FALUN SWEDEN

INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

I Definition of parameters in a histopathologic malignancy grading system

U STENDAHL H WILLÉN and R WILLÉN

Several attempts have been made to select treatment modality according to histopathologic evaluation of tumour biopsies. In 1893 VON HANSEMAN introduced the term anaplasia to denote an absence of differentiation. He believed that the degree of anaplasia was directly related to the invasive capacity of a tumour. This principle has been followed by most authors trying to evaluate the biologic activity of squamous cell carcinoma of the uterine cervix. However the designs of the classification have been most variable.

SCHOTTLAENDER & KERMAUNER (1912) divided tumours into reife, mittelreife und unreife (mature, semi mature and immature).

BRODERS (1920, 1925, 1926, 1940) and GOELLNER (1976) classified the tumours as of high, moderate and poor differentiation according to a scale from 1 to 4.

MARTZLOFF (1923, 1928) divided the tumours into normal, transitional and fat spindle cell types.

BÖHM & ZWEIFEL (1926) used four groups based on the Schottlaender & Kermauner classification. They added keratinization and suggested the value of estimating the tumour host relationship in terms of cellular response, eosinophilia and the amount of connective tissue.

HEALY & CUTTLER (1928) distinguished 3 grades of tumours (solid, papillary and anaplastic) closely corresponding

to Martzloff's grades and Broders' grades 2 to 4.

HUEPER (1928a, b) estimated both the tumour cell population and the tumour host relationship by 20 different factors each grading from 1 to 4. The score constituting the malignancy index of tumour and correlating with survival. The parameters used were: (1) special cell type of carcinoma, (2) nucleo-cytoplasmic coefficient, (3) number of pencil cells, (4) infiltrative cell growth, (5) general type of carcinoma, (6) irregular cell size, (7) irregular cell shape, (8) distinctness in outline of cells, (9) chromatism of cytoplasm, (10) functional activity of cells, (11) irregular size of nuclei, (12) irregular shape of nuclei, (13) chromatism of nuclei, (14) hyperchromatism of nuclei, (15) number of mitoses and prophase, (16) irregularity of mitoses, (17) character of stroma, (18) vascularity of stroma, (19) type of cellular infiltration of stroma and (20) amount of cellular infiltration of stroma.

WARREN (1931) and GATES & WARREN (1934) classified the carcinomas as low, medium and high malignant.

CHAMBERS (1935) grouped the tumours into adult spindle and transitional types with the following subgroups: keratinized, differentiated, transitional and anaplastic. The latter divided into an alveolar

and in solid form ACKERMAN & DEL REGATO (1947) used a histologic grading from 1 to 3 (high and moderate differentiation and an anaplastic type TRUELSEN (1949) divided the squamous cell carcinomas into parakeratotic squamous cell mucosal undifferentiated and basal cell types

GLUCKSMANN & SPEAR (1945) GILMOUR et coll (1949) classified the tumours as anaplastic (A tumours) and squamous cell differentiating tumours (D tumours) In 1951 PENDL submitted his elaborate classification with 16 different subtypes

A Epidermoid carcinoma

(I) Differentiated epidermoid carcinoma

(I) Cutaneous type (a) with horny lamellae (b) predominantly with polygonal cells (c) predominantly with spindle shaped (basal) cells

(II) Mucosal type (a) with all layers (*) differentiated as usual (**) with Holzermarkzellen (b) one layer predominating (*) predominantly spindle shaped (basal) cells (**) predominantly pale cells (***) cuboid columnar type of cell without gland formation (****) cuboid columnar type of cell with gland formation

(2) Only slight differentiated epidermoid carcinoma (a) small celled intermediate type (b) large celled intermediate type

B Anepidermoid carcinoma

(1) Transitional cell carcinoma

(2) Undifferentiated carcinoma

C Mixed type

D True adenocarcinoma

E Special types of carcinoma

REAGAN et coll (1957) and REAGAN & WENTZ (1967) classified the tumours into large cell non keratinizing large cell keratinizing and small cell carcinoma a classification adopted by WHO (POULSEN et coll 1975) GRAHAM & GRAHAM (1962) divided the tumours according to cytologic smears before and after radiation therapy into one group with good sensitization response (good SR) and the other with poor sensitization response (poor SR) A parabasal a keratinizing a pleomorphic and a small cell type grouping was used by TWEEDALE & RODDICK (1969)

Finally SEKIMOTO et coll (1978a b) designed a classification based on combination of maturation functional differentiation and cell type The latter was

divided into basal squamous prickly pleomorphic reserve and spindle cell type

The intention of all these proposed classification was to make it possible to predict the prognosis of the individual patient with fair certainty However the results varied and were often directly conflicting Some authors found a good correlation to survival (for review see LANGE 1960) others denied that histologic grading had any prognostic value (LANGE FIELD et coll 1964 NG & ATKIN 1973 GOELLNER & JOHANSSON et coll 1976) For the individual patient it was stated that no consistent guidelines as regards the future treatment of the patients could be based on the histologic appearances alone (JOHANSSON et coll)

The light microscopic technique has been complemented with immunofluorescence and immunoperoxidase methods (BONFIGLIO & FEINBERG 1976 PERTSCHUK et coll 1977 RUBIO et coll 1978) and scanning electron microscopy (RUBIO & KRANTZ 1976 RUBIO & EINHORN 1977) Calculation of DNA content (ATKIN 1964 NØDSKOV PEDERSEN 1971) and chromosome analyses (ATKIN & BAKER 1979) have been made Computer analysis of exfoliated cells from the uterine cervix (HOLMQUIST et coll 1978) and computer aided cluster and discriminant analyses (KRAMER et coll 1974) have also been attempted These methods are still not easily accessible but the aim has been to find objective and reproducible diagnostic criteria

At the Department of Tumour Pathology of Radiumhemmet Stockholm a malignancy grading system of parameters each graded in 1 to 4 points was developed to evaluate the biologic activities of squamous cell carcinoma of the larynx (JAKOBSSON 1973 1975) palate (ENEROTH & MOBERGER 1973) gingiva (WILLÉN et coll 1975) This system was used by LUND et coll (1975a b 1976 1977) in carcinomas of the lip tongue and larynx and by FISHER (1975) and HELWEG LARSEN et coll (1978) for the larynx The present malignancy grading system was adopted and modified by STENDAHL et coll (1979) for squamous cell carcinoma of the uterine cervix (8 parameters each graded from 1 to 3 points one new parameter introduced) In a retrospective investigation a high correlation was found between the sum of malignancy points and the clinical outcome of the individual The present report gives a definition and a closer description of the parameters used

Table 1

Parameters used for malignancy point grading, Tumour cell population

Parameter	Points		
	1	2	3
Structure	Exophytic papillary and solid	Small cords and groups of cells	Marked cellular dissociation
Differentiation into cell type	Large cell no keratinization	Large cell keratinized	Small cell no keratinization
Nuclear polymorphism	>75 per cent mature nuclei few enlarged nuclei	75 to 75 per cent mature nuclei moderate number of enlarged nuclei	<75 per cent mature nuclei numerous irregular or anaplastic enlarged nuclei
Mitoses	Single 0-1	Moderate number 0-5	Numerous 0-5

Table 2

Parameters used for malignancy point grading Tumour host relationship

Parameter	Points		
	1	2	3
Mode of invasion	Well defined borderline	Cords less marked borderline	Groups of cells or diffuse growth
Stage of invasion	(Min stroma inv) or microcarcinoma	Nodular into submucosa and connective tissue	Massive amongst muscles and vessels
Vascular invasion	None	Possible	Well established within the lumina of lymph or blood vessels
Cellular response (plasmolymphocytic)	Marked (continuous rim)	Moderate (several large patches)	Slight or none (few small patches or no cells)

Methods

The tumour cell population and the tumour-host relationship were considered separately. The relation of the tumour cell population (Table 1) based on the grading of cell differentiation, structure, nuclear polymorphism and the frequency of mitotic figures in terms of a 1 to 3 point scale. The tumour-host relationship (Table 2) was also estimated in terms of a 1 to 3 point scale by the mode of invasion, the stage of invasion, vascular invasion and the degree of lymphoplasmocytic infiltration. Since 8 morphologic parameters permitted a grade with 8 to 24 points totally.

Comments

Tumour cell population

Structure (parameter 1) Magnification (4×12.5) It is important that the biopsy also includes stroma. **1 point** Papillary and solid types growing exophytically. Care should be taken that it is not an inverted growth with cords and groups of cells that penetrate the stroma (Fig. 1).

2 points Small cords and groups of cells with maintained cohesion. This group also includes aggregations of 15 to 20 cells sometimes combined with mesenchymal tissue (Fig. 2).

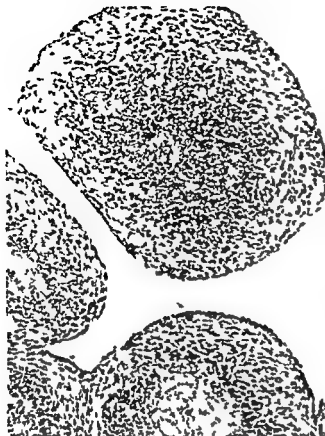


Fig 1



Fig 2



Fig 3

Fig 1 Structure (parameter 1) Papillary type One point

Fig 2 Structure (parameter 1) Small cords and groups of cells with retained cohesion Two points

Fig 3 Structure (parameter 1) Marked cellular dissociation Three points



Fig 4

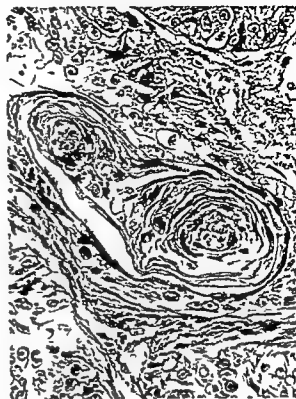


Fig 5

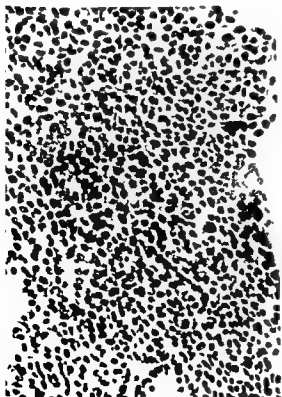


Fig 6

Fig 4 Differentiation into cell type (parameter 2) Large cell non-keratinizing type One point

Fig 5 Differentiation into cell type (parameter 2) Large cell keratinizing type with pearl formation Two points

Fig 6 Differentiation into cell type (parameter 2) Small cell non keratinizing type with marked hyperchromatism Three points



Fig 7

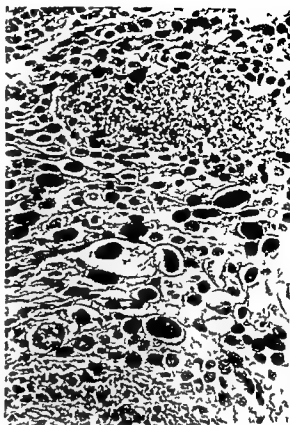


Fig 8

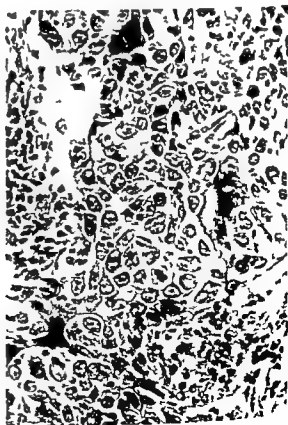


Fig 9

Fig 7 Nuclear polymorphism (parameter 3) Only slight nuclear variation with more than 75 per cent mature nuclei One point

Fig 8 Nuclear polymorphism (parameter 3) Less than 75 per cent mature nuclei with solitary giant tumour cells and a moderate number of large nuclei Two points

Fig 9 Nuclear polymorphism (parameter 3) Less than 25 per cent mature cells with numerous giant tumour cells also bizarre containing tumour cell forms Three points



Fig 10



Fig 11



Fig 12

Fig 10 Mode of invasion (parameter 5) Invasive squamous cell carcinoma with a defined borderline and clearly demarcated interface towards the stroma One point

Fig 11 Mode of invasion (parameter 5) Less distinct borderline with disintegrating cords but the cells are still connected to each other Two points

Fig 12 Mode of invasion (parameter 5) Strongly disintegrated appearances with individual cells or small cell groups Three points

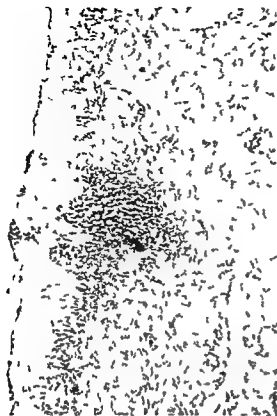


Fig 13



Fig 14



Fig 15

Fig 13 Stage of invasion (parameter 6) Slight invasion into stroma One point

Fig 14 Stage of invasion (parameter 6) Invasive squamous cell carcinoma with growth into stroma and collagen tissue not reaching the medium sized vessels or the muscles Two points

Fig 15 Stage of invasion (parameter 6) Growth close to and between medium sized vessels and also growth into the musculature Three points



Vascular invasion (parameter 7) Invasive squamous cells within the vessel wall (→) but no evidence of intra-manifestations Two points



7 Vascular invasion (parameter 7) Well established growth of tumour cells within the lumen of a blood vessel Three points

3 points Marked cellular dissociation Only a few cells may cling together (Fig 3)

Differentiation into cell type (parameter 2) follows the definition given by WHO (POULSEN et coll) Generally only one cell type is found but in mixtures of several kinds for example large aggregations of basaloid cells in combination with large cell formations points are given to the lowest differentiated form (STENDAHL et coll)

1 point Large cell non keratinizing type This is the most frequent sub-type Keratinization is absent or limited to isolated cells Sometimes these tumours are composed of cells with abundant clear cytoplasm (Fig 4)

2 points Large cell keratinizing type This sub-type is characterized by tumour cells with pearl formation Strongly keratinized parakeratotic forms are also included in this group even if only small amounts of keratine are found in intermediate and basal cell layers One to 3 cell layers with negligible keratinization in the surface area are classified with 1 point (Fig 5)

3 points Small cell non keratinizing type This type is rare (2 to 10 per cent) No keratinization is found The cells are smaller but the relative nuclear size is increased Hyperchromatism of the nuclei is marked (Fig 6)

Nuclear polymorphism (parameter 3) This is a modification of Broders system (BRODERS 1920 1925 1926 1940) Thirty high power fields are examined (40×12.5)

1 point Requires more than 75 per cent mature nuclei and only negligible size variations (Fig 7)

2 points Requires 25 to 75 per cent mature nuclei solitary giant tumour cells and a moderate number of enlarged nuclei (Fig 8)

3 points Requires less than 25 per cent mature cells extremely irregular often numerous giant tumour cells and bizarre tumour cell forms Extremely immature cells of basaloid type are included (Fig 9)

Mitoses (parameter 4) Magnification (40×12.5) This parameter requires very accurate classification Thirty high power fields are examined The counting must not only be related to the basal cell layer but also to the prickle-cell and superficial layers Clear chromosome pattern should be distinguishable to avoid interpreting hyperchromatic cell nuclei or pycnotic cell nuclei as mitoses

1 point 0 or 1 mitosis per high power field

2 points 2 to 5 mitoses per high power field



Fig 18 Lymphoplasmacytic cell response (parameter 8) Marked cellular reaction around and within the tumour nodules. One point



Fig 19 Lymphoplasmacytic cell response (parameter 8) Unevenly distributed aggregates of lymphoplasmacytic cells in some areas nearly no lymphocytes. Two points

3 points More than 5 mitoses per high power field

Tumour-host relationship

Mode of invasion (parameter 5): Magnification (10×12.5). Attention should be paid to fixation artefacts, mechanical fragmentation and some disintegration because of an inflammatory reaction in connection with intensive lymphoplasmacytic or lichenoid reaction.

1 point Defined border line with a pushing appearance where the interface continues to be clearly demarcated. When border line is slightly less distinct on account of an inflammatory reaction covering some cell layers the tumour is included in this group (Fig 10).

2 points Less distinct border line. The carcinoma penetrates the stroma with pointed disintegrating cords, still connected to each other (Fig 11).

3 points Strongly disintegrated appearance. Individual cells or groups up to 10 cells. Round aggregates containing 10 to 15 cells or more, often with a marked hyaline membrane, are given 1 point (Fig 12).

Stage of invasion (parameter 6) The biopsy should include all cell layers also including the lamina muscularis.

1 point Minimum stromal invasion or microcarcinoma is defined according to WHO (POULSEN et coll.). Minimal stromal invasion is carcinoma in situ with areas giving suspicion of penetration into the underlying stroma. Microcarcinoma is defined as stromal invasion limited to isolated microscopic foci (generally 5 mm or less in depths) (Fig 13).

2 points Growth into stroma and collagen tissue where the tumour should not penetrate deeper than one vessel diameter from vessels of medium size. The border line between 2 and 3 points may sometimes be difficult to detect. A deep biopsy and accurate examination of tumour growth in relation to the medium sized vessels is necessary (Fig 14).

3 points Growth close to medium sized vessels or between the vessels and growth into the musculature (Fig 15).

Vascular invasion (parameter 7) This parameter is difficult to estimate. Fixation artefacts and mechanical fragmentation that easily give spaces with intima like covering must be identified.



Fig. 0 Lymphoplasmocytic cell response (parameter 8) Slight cell reaction in the stroma. No reaction within the tumour nodules. Three points

- 1 point No vascular invasion
- 2 points Growth around vessels with compressive effect or infiltrating the wall without reaching the lumen (Fig. 16)
- 3 points Clear and well established growth of tumour cells into the lumen of lymphatics or blood vessels (Fig. 17)

Lymphoplasmocytic cell response (parameter 8)
Magnification (10×12.5)

- 1 point Marked cellular reaction with a continuous border of lymphoplasmocytic cells around the tumour nodules in many places (Fig. 18)
- 2 points Unevenly distributed aggregates of lymphoplasmocytic cells other areas sparsely covered with lymphocytes (Fig. 19)
- 3 points Minimum or no lymphoplasmocytic cell reaction e.g. solitary small clusters minimum amount of dissociated cells or no lymphoplasmocytic cells at all (Fig. 20)

Discussion

Microscopic classification of squamous cell carcinoma was presented by VON HANSEMAN, SCHOTTLAENDER & KERMAUNER and BRODEKS

(1920 1925 1926 1940) mainly as a monofactorial system based on cellular differentiation. A number of similar systems have later followed (for review see LANGE).

It was early realized that the connective tissue had a capacity to prevent progression of the tumour and the tumour-host relationship was carefully analysed. SCHOTTLAENDER & KERMAUNER described different tumour-connective tissue relations: medullary tumours in which the connective tissue made up a small part; a scirrhous type with preponderance of connective tissue; and finally mixed types. Scirrhous tumours are rather uncommon and were considered to offer the best prognosis. ADLER (1916) stated that apart from the type of connective tissue the vascularity of the tumour might influence the prognosis. He gave 4 possible combinations of which tumours rich in connective tissue and sparsely vascularized were supposed to have the best prognoses following radiation therapy.

LAHM (1927) divided the connective tissue reaction around the tumour into 4 grades: Grade 1 hyperaemia and round-cell infiltration; grade 2 granulation tissue in the interface between tumour parenchyma and the underlying connective tissue; grade 3 no cellular reaction; and grade 4 sclerotic connective tissue. Prognosis was said to be best in grade 4. LAX (1950) classified the connective tissue reaction into 3 groups: (1) satisfactory new formation of connective tissue with regular arrangement and ample fibres; (2) poor reaction with no or little formation of fibres; and (3) shrinkage, hyalinization and partial breakdown of the connective tissue with rupture and splitting of the fibres. In this system prognosis deteriorated from 1 to 3. LINELL & MÄNSSON (1952) evaluated the amount and type of stroma but found no relation to survival. PAAVOLAINEN (1970) and PAAVOLAINEN *et al.* (1973) found an increased frequency of local recurrences when acid mucopolysaccharides increased in the stroma.

Contrary to LAHM, SCHOCK (1926) and STUPER (1953a, b), LINELL & MÄNSSON could not find that a large number of eosinophilic cells was correlated to better prognosis.

An increased number of granulocytes resulted in decreased survival (HUEPER & SCHWITZ 1927; HUEPER 1928a, b). Tumour necrosis was also correlated to higher mortality (KOTTMEIER 1955; GLUCKSMANN & SPEAR). An enhancement of lymphocytes into the stroma was said to reduce the

frequency of metastasis (CHERRY & GLUCKSMANN 1955 GUSBERG et coll 1971)

BÖHM & ZWEIFEL LINELL & MÄNSSON as well as CULLHED (1978) reported that absence of lymphocytic infiltration was an unfavourable prognostic sign of survival However BARBER et coll (1978) found no such correlation CULLHED likewise demonstrated a significant low frequency of lymph gland metastasis when ■ high rate of lymphocytes around the tumour complexes was encountered The growth of the carcinoma into the underlying stroma was observed with attention paid to the mode of infiltration Tumours with ribbon and bandlike configurations had a better clinical outcome than those with complete cellular dissociation (SCHOTTLAENDER & KERMAUNER STÜPER 1953 a b LAX BEECHAM et coll 1978 CULLHED)

The importance of vascular invasion is evident and patients with such a feature face worse clinical outcome in both operated and irradiated patients (STEIN WERBLOWSKY 1955 CHERRY & GLUCKSMANN BARBER et coll CULLHED)

Several multifactorial classification systems have been developed HUEPER (1928 a b) devised an index of malignancy by which the sensitivity of the tumour to radiation could be assessed It was claimed to correlate well with survival The complicated classification giving points for 20 different factors made the system highly subjective and has not become widely used PENDL's classification ■ very elaborate but requires a large material of patients Groups 2 3 6 and 13 comprise 60 to 65 per cent of all patients (PENDL SCHULLER 1952 STÜPER 1953 a b) and the other 11 groups constitute the remaining 35 to 40 per cent In STÜPER ■ large series of 815 patients 9 of the groups comprised less than 40 patients and 4 less than 10 patients However well founded the PENDL classification may be morphologically and theoretically it does not appear to be suitable for routine use

SCHULLER demonstrated that PENDL's classification could also be used when reaching decision whether a patient should be operated upon or irradiated CULLHED used several of the parameters used in the present material but made no attempt to calculate total malignancy points The functional classification of SEKIMOTO et coll (1978 a b) has been published in short communications only The clinical value remains to be proven

The present malignancy grading system includes a description of the tumour cell population and the

tumour-host relationship and is an attempt to create a simple and reproducible method The parameters were chosen on the basis of the good results previously obtained by ENEROTH & MOBERGER WILLÉN et coll LUND et coll (1975 a b 1976 1977) CULLHED and STENDAHL et coll but these parameters will not necessarily be those finally used The prognostic value of the parameters may also be revised A multifactorial analysis is at present in progress which will provide further information in this respect Preliminary results indicate that most of the prognostic information ■ found in the tumour-host relationship

It is essential that all parameters can be identified in the biopsy and that characteristics of the invasive zone are not underestimated This ■ in agreement with JAKOBSSON (1973) but is contrary to the results of LUND et coll (1975 a b 1976 1977) and HELWEG LARSEN et coll thus implying that large and deep biopsies must be taken in order to fit into the grading system The biopsy should also be large in order to reduce the sampling error ■ parameters such as cell type nuclear polymorphism mitoses and mode of invasion may be unevenly distributed in the tissue (MARTZLOFF 1928 SILVERBERG 1976 CHI et coll 1977)

SUMMARY

A histologic malignancy grading system for invasive squamous cell carcinoma of the uterine cervix is presented The method is based upon evaluation of the tumour cell population in terms of cell differentiation structure nuclear polymorphism and the frequency of mitotic figures in terms of a 1 to 3 point scale The tumour host relationship was also estimated in terms of a 1 to 3 point scale by the mode of invasion stage of invasion vascular invasion and degree of lymphoplasmocytic infiltration These parameters permitted a grading with 8 to 24 points totally

ACKNOWLEDGEMENTS

Financial support was received from Stockholms Cancerförening The authors wish to thank Bengt Holmquist and Nils Lindgren for expert library service and Gösta Andersson for photography

REFERENCES

- ACKERMAN L V and DEL REGATO J A Cancer Diagnosis treatment and prognosis First edition C V Mosby St Louis 1947

- ER L Morphologische Kennzeichen für die Radiempfindlichkeit der Karzinome des weiblichen Genitals *Zbl Gynak* 40 (1916) 673
- EVAN N Nuclear size in carcinoma of the cervix Its relation to DNA content and to prognosis *Cancer* 17 (1964) 1391
- FRANKLIN BAKER M C Chromosome 1 in 26 carcinomas of the cervix uteri Structural and numerical changes *Cancer* 44 (1979) 604
- FRANKLIN H K K SOMMERS S C RATTERDAM H and JACOBSON T Vascular invasion as a prognostic factor in stage I II cancer of the cervix *Obstet and Gynec* 52 (1978) 343
- FRANKLIN J H HALVORSEN T and KOLBENSTVEDT A Histologic classification lymph node metastases and distant survival in stage I B cervical carcinoma An analysis of 245 uniformly treated cases *Gynec Oncol* (1978) 95
- FRANKLIN D and ZWEIFEL E Inwieweit kann man heute aus makroskopischen Befunden eine Prognose für die Behandlung des Uteruskarzinoms stellen? *Zbl Gynak* 1 (1926) 30
- FRANKLIN T A and FEINBERG M R Isoantigen loss in cervical neoplasia Demonstration by immunofluorescence and immuno-peroxidase techniques *Arch Path Lab Med* 100 (1976) 307
- FRANKLIN A C Squamous cell epithelioma of the lip *J Amer med Ass* 74 (1920) 656
- FRANKLIN The grading of carcinoma *Minn Med* 8 (1925) 726
- FRANKLIN Carcinoma grading and practical application *Arch Path (Chic)* 2 (1926) 376
- FRANKLIN Microscopic grading of cancer *In Treatment of cancer and allied diseases* 9 Edited by G T Pack and E M Livingstone Paul B Hoeber New York 1940
- FRANKLIN H The histological classification of cancers of the uterine cervix and the relation between the growth structure and the results of radium treatment *Amer J Cancer* 23 (1935) 1
- FRANKLIN RRY C P and GLUCKSMANN A Lymphatic embolism and lymph node metastasis in cancers of vulva and of uterine cervix *Cancer* 8 (1955) 564
- FRANKLIN C H RUBIO C A and LAGERLOF B The frequency and distribution of mitotic figures in dysplasia and carcinoma in situ *Cancer* 39 (1977) 1218
- FRANKLIN LHED S Carcinoma cervicis uteri Stages I and II A Treatment-histopathology-prognosis Thesis Linköping 1978
- FRANKLIN ROTH C M and MOBERGER G Histological malignancy grading of squamous cell carcinoma of the palate *Acta oto-laryng (Stockh)* 75 (1973) 293
- FRANKLIN LD D A DOCKERTY M B and SYMONDS R E Small cell cancer of the cervix *Amer J Obstet Gynec* 88 (1964) 447
- FRANKLIN HER H H Grading of biopsies of laryngeal carcinoma by multiple criteria *Canad J Otolaryng* 4 (1975) 881
- FRANKLIN TES O and WARREN S The grading of epidermoid carcinoma *Surg Gynec Obstet* 58 (1934) 962
- FRANKLIN MOUR M GLUCKSMANN A and SPEAR F G The influence of tumour histology duration of symptoms and age of patients on the radiocurability of cervix tumours *Brit J Radiol* 22 (1949) 90
- FRANKLIN GLUCKSMANN A and SPEAR F G The qualitative and quantitative histological examination of biopsy material from patients treated by radiation for carcinoma of the cervix uteri *Brit J Radiol* 18 (1945) 313
- FRANKLIN GOELLNER J R Carcinoma of the cervix Clinical pathologic correlation of 196 cases *Amer J Clin Path* 66 (1976) 775
- FRANKLIN GRAHAM R M and GRAHAM J B Cytologic prognosis in cancer of cervix Two year survival rates in randomized series *Amer J Roentgenol* 87 (1962) 56
- FRANKLIN GUSBERG S B YANNOPOULOS A and COHEN C J Virulence indices and lymph nodes in cancer of the cervix *Amer J Roentgenol* 111 (1971) 273
- FRANKLIN VON HANSEMAN D Studien über die Spezifität des Altruismus und die Anaplasia der Zellen A Hirschwald Berlin 1893
- FRANKLIN HEALY W and CUTTLER M Relation between structure and prognosis in cervical carcinoma under radiation treatment *Amer J Obstet* 16 (1928) 15
- FRANKLIN HELWEG-LARSEN K GRAEM N MEISTRUP LARSEN K I and MEISTRUP-LARSEN U Clinical relevance of histological grading of cancer of the larynx *Acta path microbiol scand Sect A* 86 (1978) 499
- FRANKLIN HUEPER W C (a) The relation of the histological structure to the prognosis of the carcinomas of the uterine cervix *Surg Gynec Obstet* 47 (1928) 502
- FRANKLIN — (b) Carcinomas of the uterine cervix Their histologic structure malignancy and prognosis *Arch Path* 6 (1928) 1064
- FRANKLIN — and SCHWITZ H Der histologische Malignitätsindex und seine Bedeutung für Prognose und Behandlung der Cervixcarcinome des Uterus *Strahlentherapie* 24 (1927) 660
- FRANKLIN — Der prognostische Wert des histologischen Malignitätsindex und der klinischen Einleitung der Cervixcarcinome des Uterus *Strahlentherapie* 30 (1928) 650
- FRANKLIN HOLMQUIST J BENGTSSON E ERIKSSON O NORDIN H and TENKVIST H Computer analysis of cervical cells Automatic feature extraction and classification *J Histochem Cytochem* 26 (1978) 1000
- FRANKLIN JAKOBSSON P Å Glottic carcinoma of the larynx Factors influencing prognosis following radiotherapy Thesis Karolinska Institutet Stockholm 1973
- FRANKLIN — Histologic grading of malignancy and prognosis in glottic carcinoma of the larynx *Canad J Otolaryng* 4 (1975) 885
- FRANKLIN JOHANSSON O JOHANSSON J E LINDBERG L G and SYDÖ A Prognosis recurrences and metastases correlated to histologic cell type in carcinoma of the uterine cervix *Acta obstet gynec scand* 55 (1976) 255
- FRANKLIN KOTTMEIER H L The place of radiation therapy and of surgery in the treatment of uterine cancer *J Obstet Gynaec Brit Emp* 62 (1955) 737
- FRANKLIN KRAMER I R H EL LABBAN N G and SONKOP E Further studies on lesions of the oral mucosa using computer aided analyses of histological features *Brit J Cancer* 29 (1974) 223

- LAHM W Die Bedeutung der mikroskopischen Untersuchung für die Behandlung und Prognose des Collumkarzinoms *Ergebn Strahlenforsch* 1 (1927) 556
- LANGE P Clinical and histopathological studies on cervical carcinoma *Acta path microbiol scand* 50 (1960) Suppl No 143
- LAX H Die Prognose der Kollumkarzinome auf Grund histologischer Beurteilung *Zbl Gynak* 72 (1950) 284
- LINELL F and MÄNSSON B Prognostic value of histologic grading of carcinoma of cervix uteri. A study of 388 cases treated with radium and roentgen therapy *Acta radiol* 38 (1952) 219
- LUND C SØGAARD H JØRGENSEN K and HIJELM HANSEN M Epidermoid carcinoma of the larynx IV Histologic grading in the clinical evaluation *Acta radiol Ther Phys Biol* 15 (1976) 293
- ELBROND O JØRGENSEN K and ANDERSEN A P (a) Epidermoid carcinoma of the lip Histologic grading in the clinical evaluation *Acta radiol Ther Phys Biol* 14 (1975) 465
- — — — (b) Epidermoid carcinoma of the tongue Histologic grading in the clinical evaluation *Acta radiol Ther Phys Biol* 14 (1975) 513
- JØRGENSEN K ELBROND O HIJELM HANSEN M and ANDERSEN A P Histologic grading of epidermoid carcinomas in the head and neck *Danish med Bull* 24 (1977) 162
- MARTZLOFF K H Carcinoma of cervix uteri Pathological and clinical study with particular reference to relative malignancy of neoplastic process as indicated by predominant type of cancer cell *Bull Johns Hopk Hosp* 34 (1923) 141
- Epidermoid carcinoma of cervix uteri Histologic study to determine resemblance between biopsy specimens and parent tumour obtained by radical panhysterectomy *Amer J Obstet Gynec* 16 (1928) 578
- NG A II P and ATKIN N B Histological cell type and DNA value in the prognosis of squamous cell cancer of uterine cervix *Brit J Cancer* 28 (1973) 322
- NODSKOV PEDERSEN S Degree of malignancy of cancer involving the cervix uteri judged on the basis of clinical stage histology size of nuclei and content of DNA *Acta path microbiol scand Sect A* 79 (1971) 617
- PAAYOLAINEN M Stromal reactions as prognostic factors in epidermoid carcinoma of the larynx Thesis *Hel sinki* 1970
- TARKKANEN J and SAKSELA E Stromal reactions as prognostic factors in epidermoid carcinoma of the tongue *Acta oto laryng (Stockh)* 75 (1973) 316
- PENDL O Histologische Klassifizierung und Ergebnisse der Strahlenbehandlung des Carcinoma colli uteri *Radiol austriaca* 4 (1951) 95
- PERTSCHUK L P BOYCE J G and URCUYO R An immunofluorescent study of basement membranes in squamous cell carcinoma of the cervix vagina and vulva *Obstet and Gynec* 49 (1977) 417
- POULSEN II E TAYLOR C W and SOBIN L II Histological typing of female genital tract tumours *In International histological classification of tumours* No 13 WHO Geneva 1975
- REAGAN J W and WENTZ W B Genesis of carcinoma uterine cervix *Clin Obstet Gynec* 10 (1967) 883
- HARMONIC M J and WENTZ W B Analytical study of cells in cervical squamous cell cancer *Lab Invest* (1957) 241
- RUBIO C A and EINHORN N The exfoliating epithelial surface of the uterine cervix IV Scanning electron microscopic study in invasive squamous carcinoma of human subjects *Beitr Path* 161 (1977) 72
- and KRANZ I The exfoliating cervical epithelial surface in dysplasia carcinoma in situ and invasive squamous carcinoma I Scanning electron microscopic study *Acta cytol* 20 (1976) 144
- BIBERFELD P and EINHORN N The immunofluorescence characteristics of the basement membrane of squamous carcinoma of the uterine cervix *Histopathology* 2 (1978) 67
- SCHÖCH E U Über die lokale Eosinophilie bei Karzinom *Zbl Gynak* 45 (1926) 2895
- SCHOTTLEANDER J and KERMAUNER F Zur Kenntnis des Uterus Karzinoms S Karger Berlin 1912
- SCHULLER E Beziehungen des histologischen Tumortypus des Collumcarcinoms zur Dauerheilung durch Operation und seine Bedeutung für Prognose und Therapie dieser Erkrankung *Arch Gynak* 181 (1957) 36
- SEKIMOTO K NAKANO M and SATOH S (a) Sequential cytopathological changes of epidermoid carcinoma of the uterine cervix I Comparison between type bas and type sqm *J Jap Cancer Clin* 24 (1978) 203
- TANAKA N and KENO T (b) Sequential cytopathological changes following irradiation of epidermoid carcinoma of the uterine cervix of spindle cell type in our classification *J Jap Cancer Clin* 24 (1978) 603
- SILVERBERG S G Reproducibility of the mitosis count in the histologic diagnosis of smooth muscle tumours of the uterus *Human Path* 7 (1976) 451
- STEN WERBLOWSKY R Prognosis in cancer of the cervix uteri *Brit J Radiol* 28 (1955) 673
- STENDAHL U WILLEN H and WILLEN R Classification and grading of invasive squamous cell carcinoma of the uterine cervix *Acta radiol Oncology* 18 (1979) 481
- STUPER P (a) Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome I Mitteilung Untersuchungen über die histologische Klassifizierung nach Pendl *Strahlentherapie* 92 (1953) 89
- (b) Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome 4 Mitteilung Untersuchungen zur Frage der elektiven Therapie *Strahlentherapie* 92 (1953) 338
- TRUELSEN F Cancer of the uterine cervix Rosenkilde and Bagger Copenhagen 1949
- TWEEDALE D N and RODDICK JR J W Histologic types of squamous cell carcinoma in situ of the cervix *Obstet and Gynec* 33 (1969) 35
- WARREN S Grading of carcinoma of the cervix as checked at autopsy *Arch Path (Chic)* 12 (1931) 783
- WILLEN R NATHANSSON A MOBERGER G and ANDERSON G Squamous cell carcinoma of the gingiva Histological classification and grading of malignancy *Acta oto laryng (Stockh)* 79 (1975) 146

SURVIVAL IN CARCINOMA OF THE UTERINE CERVIX CORRELATED TO PRIMARY TREATMENT RESULTS

J. JIMENEZ, J. ALERT, L. BELDARRAÍN
J. MONTALVO and C. ROCA

Carcinoma of the uterine cervix is clinically generally confined to the pelvis although distant metastases to lung, liver, bones etc. may occur. Many patients die without signs of local activity. Thus AD et coll. (1979) found tumor spread into the pelvis and abdomen in patients with an apparently in situ cervix after radiation therapy. In a group of patients with cervical carcinoma the survival was correlated to the effect of the irradiation at the end of the radiation therapy and the result was presented.

Material and Methods

The material consisted of 1106 patients with carcinoma of the uterine cervix irradiated between 1967 and 1972 at this institute. The method of treatment has been reported previously (JIMENEZ et coll. 1979). The cases were classified according to M. 186 patients (16.9%) belonged to stage I, 81 (7.3%) to stage IIa, 608 (54.9%) to stage IIb, 176 (15.9%) to stage III and 55 (4.9%) to stage IV.

Results

The 5 year survival rate and the result at the end of radiation appear in the Table. To clinical evidence of tumor activity was found in 154 patients (77.2%). 571 (66.8%) were alive and 115 years after treatment.

Doubtful tumor activity was considered in 135 patients (12.2%). 47 (34.8%) were alive and well 5 years after treatment.

Evident tumor activity was considered to be present in 117 patients (10.6%) at the end of irradiation. 18 (15.4%) were alive and without signs of activity 5 years after treatment. 8 of these belonged to stage III and 3 to stage IV.

Discussion

At the end of the irradiation the tumor may have disappeared completely at clinical examination. In other cases evident tumor tissue remains and in still another group it is doubtful whether viable tumor tissue remained. When the 5 year survival is analysed the clinical evaluation of the tumor at the end of irradiation does not give a reliable indication of the long term survival.

Cytology may give an erroneous result in case of recurrence under a healthy epithelium (KRAUS & SCHUHMAN 1979). It is also possible that in a biopsy specimen small areas of viable cells may be overlooked at microscopy (PILLERON et coll. 1972).

In stage I the survival figures decrease when the clinical examination gives doubtful results or an evident tumor is found. The same is valid in stages IIa and IIb.

- LAHM W Die Bedeutung der mikroskopischen Untersuchung für die Behandlung und Prognose des Collumkarzinoms *Ergebn Strahlenforsch* 1 (1927) 556
- LANGE P Clinical and histopathological studies on cervical carcinoma *Acta path microbiol scand* 50 (1960) Suppl No 143
- LAX H Die Prognose der Kollumkarzinome auf Grund histologischer Beurteilung *Zbl Gynak* 72 (1950) 284
- LINELL F and MÅNSSON II Prognostic value of histologic grading of carcinoma of cervix uteri A study of 388 cases treated with radium and roentgen therapy *Acta radiol* 38 (1952) 219
- LUND C SOGAARD H JØRGENSEN K and HJELM HANSEN M Epidermoid carcinoma of the larynx IV Histologic grading in the clinical evaluation *Acta radiol Ther Phys Biol* 15 (1976) 293
- — — — — ELBRÖD O JØRGENSEN K and ANDERSEN A P (a) Epidermoid carcinoma of the lip Histologic grading in the clinical evaluation *Acta radiol Ther Phys Biol* 14 (1975) 465
- — — — — (b) Epidermoid carcinoma of the tongue Histologic grading in the clinical evaluation *Acta radiol Ther Phys Biol* 14 (1975) 513
- — — — — JØRGENSEN K ELBRÖD O HJELM HANSEN M and ANDERSEN A P Histologic grading of epidermoid carcinomas in the head and neck *Danish med Bull* 24 (1977) 162
- MARTZLOFF K H Carcinoma of cervix uteri Pathological and clinical study with particular reference to relative malignancy of neoplastic process as indicated by predominant type of cancer cell *Bull Johns Hopk Hosp* 34 (1923) 141
- Epidermoid carcinoma of cervix uteri Histologic study to determine resemblance between biopsy specimens and parent tumour obtained by radical panhysterectomy *Amer J Obstet Gynec* 16 (1928) 578
- NG A B P and ATAIN N B Histological cell type and DNA value in the prognosis of squamous cell cancer of uterine cervix *Brit J Cancer* 28 (1973) 322
- NODSKOV PEDERSEN S Degree of malignancy of cancer involving the cervix uteri judged on the basis of clinical stage histology size of nuclei and content of DNA *Acta path microbiol scand Sect A* 79 (1971) 617
- PAAVOLAINE M Stromal reactions as prognostic factors in epidermoid carcinoma of the larynx Thesis Helsinki 1970
- — — — — TARKKANEN J and SAKSELA E Stromal reactions as prognostic factors in epidermoid carcinoma of the tongue *Acta oto laryng (Stockh)* 75 (1973) 316
- PENDL O Histologische Klassifizierung und Ergebnisse der Strahlenbehandlung des Carcinoma colli uteri *Radiol austraca* 4 (1951) 95
- PERTSCHUK L P BOYCE J G and URCUYO R An immunofluorescent study of basement membranes in squamous cell carcinoma of the cervix vagina and vulva *Obstet and Gynec* 49 (1977) 417
- POULSEN H E TAYLOR C W and SOBIN L II Histological typing of female genital tract tumours *In International histological classification of tumours* No 13 WHO Geneva 1975
- REAGAN J W and WENTZ W B Genesis of carcinoma of uterine cervix *Clin Obstet Gynec* 10 (1967) 883
- — — — — HARMONIC M J and WENTZ W B Analytical study of cells in cervical squamous cell cancer *Lab Invest* 6 (1957) 241
- RUBIO C A and EINHORN N The exfoliating epithelial surface of the uterine cervix IV Scanning electron microscopical study in invasive squamous carcinoma of human subjects *Beitr Path* 161 (1977) 72
- — — — — KRANZ I The exfoliating cervical epithelial surface in dysplasia carcinoma in situ and invasive squamous carcinoma I Scanning electron microscopic study *Acta cytol* 20 (1976) 144
- — — — — BIBERFELD P and EINHORN N The immunofluorescence characteristics of the basement membrane in squamous carcinoma of the uterine cervix *Histopathology* 2 (1978) 67
- SCHOCH E U Über die lokale Eosinophilie bei Karzinom *Zbl Gynak* 45 (1926) 2895
- SCHOTTLAENDER J and KERMALNER F Zur Kenntnis des Uterus Karzinoms *M Karger* Berlin 1917
- SCHULLER E Beziehungen des histologischen Tumortypus des Collumcarcinoms zur Dauerheilung durch Operation und seine Bedeutung für Prognose und Therapie dieser Erkrankung *Arch Gynak* 181 (1957) 360
- SEKIMOTO K NAKANO M and SATOH S (a) Sequential cytopathological changes of epidermoid carcinoma of the uterine cervix I Comparison between type base and type sqm *J Jap Cancer Clin* 24 (1978) 203
- — — — — TANAKA N and KENO T (b) Sequential cytopathological changes following irradiation of epidermoid carcinoma of the uterine cervix of spindle cell type in our classification *J Jap Cancer Clin* 24 (1978) 603
- SILVERBERG S G Reproducibility of the mitosis count in the histologic diagnosis of smooth muscle tumors of the uterus *Human Path* 7 (1976) 451
- STEN WERBLOWSKY E Prognosis in cancer of the cervix uteri *Brit J Radiol* 28 (1955) 623
- STENDAHL U WILLÉN H and WILLÉN R Classification and grading of invasive squamous cell carcinoma of the uterine cervix *Acta radiol Oncology* 18 (1979) 481
- STUPER P (a) Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome I Mitteilung Untersuchungen über die histologische Klassifizierung nach Pendl *Strahlentherapie* 92 (1953) 89
- — — — — (b) Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome 4 Mitteilung Untersuchungen zur Frage der elektiven Therapie *Strahlentherapie* 92 (1953) 338
- TRUELSEN F Cancer of the uterine cervix Rosenkilde and Bagger Copenhagen 1949
- TWEEDALE D N and RODDICK JR J W Histologic types of squamous cell carcinoma in situ of the cervix *Obstet and Gynec* 33 (1969) 35
- WARREN S Grading of carcinoma of the cervix as checked at autopsy *Arch Path (Chic)* 12 (1931) 783
- WILLÉN II NATHANSSON A MOBERGER G and ANDEROTH G Squamous cell carcinoma of the gingiva Histological classification and grading of malignancy *Acta oto-laryng (Stockh)* 79 (1975) 146

IRON INCORPORATION AFTER SINGLE AND FRACTIONATED
IRRADIATION OF INFANT MICE

G II GERBER and J MAES

fractionation of a radiation dose in general per-
mits repair of radiation injury and thus causes
less injury than the same dose delivered in a single
fraction. However, certain exceptions to this rule
exist. Thus, mice exposed on day 6 and day 9 of
life play a higher mortality than when they are
exposed to the same dose either on day 6 or on day 9
(ANN & MUTH 1977). The mechanism causing
this sensitization is not yet known. It may be related
to adaptation of hemopoiesis to adult life taking
place during the neonatal period (METCALF &
MUTH 1971), a suggestion which is supported by
the fact that the sensitization ceases as the mice
reach adulthood. Since such an apparent lack of
adaptation of hemopoiesis in the infant organism is
of interest on theoretic grounds but also has im-
portant implications for radiation therapy and radia-
tion protection, it was considered of interest to elu-
cidate this mechanism in more detail. The present
paper deals with the normal development of the
hemopoietic system during the infancy of mice and the
effects caused by a single and a fractionated radia-
tion dose. Incorporation of iron into hemopoietic
cells was utilized to assess the capacity of the
hemopoietic system (BELCHER et al. 1954;
MUTH & TRIBUKAIT 1971; cf. also review in
MUTH & ALTMAN 1970). Later GERBER & MAES
(1978) reported on the behavior of the stem cells
with respect to their sensitivity to radiation as well as the sig-
nificance of such a sensitization for late effects.

Methods

Infant mice of the C₅₇Bl strain were whole body
irradiated at 250 kV, 1 mm Cu filter, 0.84 Gy/min.
The following groups were used: (1) Controls; (2) ex-
posed to 1.26 Gy on day 6; (3) exposed to 4.2 Gy on
day 6; (4) exposed to 4.2 Gy on day 9; (5) exposed
to 1.26 Gy on day 11 followed by 2.94 Gy on day 9.
At an age of 7, 8, 10, 14, 17, 20, 25 or 30 days, the
mice were injected intraperitoneally with 37 MBq of
⁵⁹Fe citrate and killed 4 hours later. The activity of
serum and erythrocytes was determined by gamma
spectrometry after separation by centrifugation in
calibrated hematocrit tubes, and the data were relat-
ed to 14 ml of blood. In addition, the dependence
on time after injection of serum and erythrocyte
activities was followed for selected doses and time
intervals after exposure. Activities of both femurs,
liver and spleen and bone marrow were also
assayed. Practically all activity is bound to the pro-
tein fraction at this time. All experimental groups
were followed in at least two independent exper-
iments. 4 to 6 animals were used for each time point.

Results

Preliminary experiments demonstrated that the
strain used showed about the same sensitization as
that of BAUMANN & MUTH. All animals survived a

dose of 1.26 Gy about 5 to 10 per cent died before an age of 30 days after a single dose of 4.2 Gy given on day 6 or 9. 50 per cent of the mice given the fractionated irradiation (1.26 Gy on day 6, 2.94 Gy on day 9) had died at an age of 20 days and 80 per cent had died before an age of 30 days.

The growth of infant mice is retarded by irradiation in a dose dependent manner. Whereas controls had attained a weight of 15 g at an age of 30 days, mice exposed to 1.26 Gy weighed 10 g and those exposed to 4.2 Gy 7.5 g on day 30. No difference being discernible between animals irradiated with a single or a fractionated dose. Similar results were observed with respect to liver or spleen weight. Hematocrit values displayed a dose dependent reduction which was more marked and longer lasting after a fractionated exposure to 4.2 Gy than after a single one (Fig. 1). ^{59}Fe in serum, i.e. that not incorporated into heme, rapidly diminished with time to low levels in the controls (not shown, half life time less than 30 min). Four hours after injection only traces of active iron were found in the serum of control mice, whereas much higher activities remained in that of irradiated mice, maximum values being observed 1 to 3 days after exposure (Fig. 2). One week after exposure, serum activity had again returned to normal, but in mice given a fractionated irradiation the serum iron rose again from an age of 20 days until day 30.

In explaining the data on iron incorporation into heme it should be recalled that after 4 hours many cells with newly synthesized heme have already left the hemopoietic tissues and have entered the blood. The time at which labelled erythrocytes appear in blood is shorter in young than in older mice and also diminishes during regeneration after irradiation. Radioactivity incorporated into heme of bone marrow (Fig. 3) increased in controls between day 15 and day 20. Irradiation with 1.26 Gy on day 6 caused a temporary increase in activity 1 to 3 days later, whereas irradiation with 4.2 Gy on day 6 reduced incorporation in bone marrow during the following week and prevented the age related rise in non irradiated mice. This is also observed following exposure to 4.2 Gy on day 9, except that the early decrease is absent. Fractionated exposure caused a marked temporary increase in activity at an age of 15 days and prevented the normal increase during the third week of life.

Iron incorporation into heme of control spleens (Fig. 4) increased slowly up to the third week of life

and then declined. This rise and decline seem to be slightly delayed after 1.26 Gy. However, irradiation with higher doses caused an immediate fall in iron incorporation, later the values approached normal.

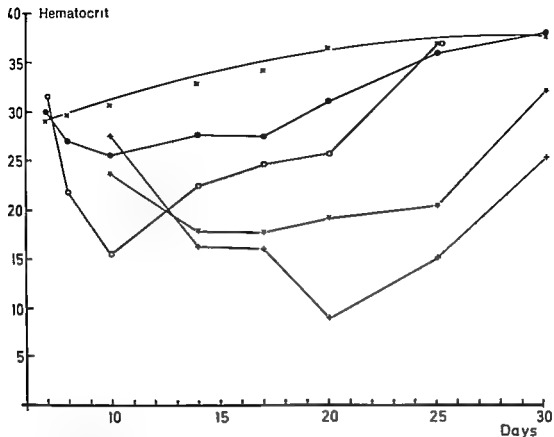
Iron incorporation into hepatic heme (Fig. 5) appeared to be nearly independent of the age of the animals. After irradiation this incorporation was increased to a degree which appears about inversely proportional to the fall in incorporation of iron into hemopoietic tissues.

The percentage of active iron appearing in erythrocytes in 0.14 ml of blood decreased with age (Fig. 6). During the initial 4 days after irradiation ^{59}Fe activity in erythrocytes diminished slightly after 1.26 Gy and markedly after 4.2 Gy. The fall after a fractionated exposure was less marked than after a single one. During the second week after exposure to 4.2 Gy erythrocyte activity was markedly enhanced above control levels and then returned to normal. The changes after a fractionated exposure, although less marked, were long lasting.

Discussion

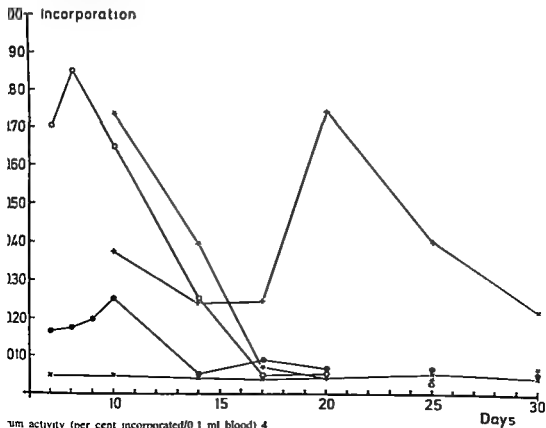
The early period after birth is characterized by profound modifications in hemopoietic functions (METCALF & MOORE). Already before birth hemopoiesis in the liver has been replaced by that in the spleen and from the second week of life, i.e. at a time when adult hematocrit levels are attained, hemopoiesis in bone marrow takes more and more precedence to that in the spleen. This is also demonstrated by the data presented. Kinetic investigations on iron incorporation into erythrocytes (not shown in the data) indicate that the time interval until labelled erythrocytes appear after ^{59}Fe injection is the maturation of heme synthesizing cells, also increases with age during the neonatal period.

In explaining the data after irradiation it should be kept in mind that only mice surviving at a given time are examined. When severely injured mice die and are thus removed from the experimental group, the mean of the surviving animals may suggest an improvement of a given parameter. Moreover, the relations between iron activity of erythrocytes and those in the hemopoietic organs must also be taken into account. The data demonstrate that irradiation during infancy produces a bone marrow syndrome characterized by profound alterations particularly in erythropoiesis. The maturation of the erythropoietic functions of bone marrow appear especially affected.



hematocrit (in per cent) of control and irradiated infant mice from age of 7 to 30 days (x controls ● 1.26 Gy on day 6 ○ 4.2 Gy on day 6 ★ 4.2 Gy on day 9 ◻ 1.26 Gy on day 6 followed by 9.4 Gy on day 9)

6 ○ 4.2 Gy on day 6 ★ 4.2 Gy on day 9 ◻ 1.26 Gy on day 6 followed by 9.4 Gy on day 9



Iron incorporation (per cent incorporated/0.1 ml blood) 4 days after injection of ^{59}Fe into control and irradiated infant mice as in Fig. 1

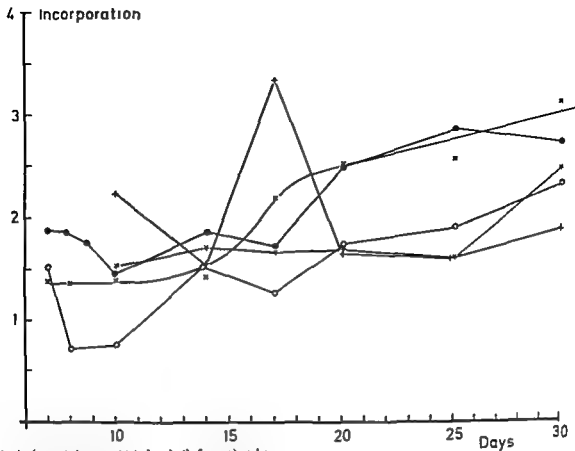


Fig 3 Activity (per cent incorporated in both femurs) in bone marrow of control and irradiated infant mice 4 hours after injection of ^{59}Fe . Symbols as in Fig 1

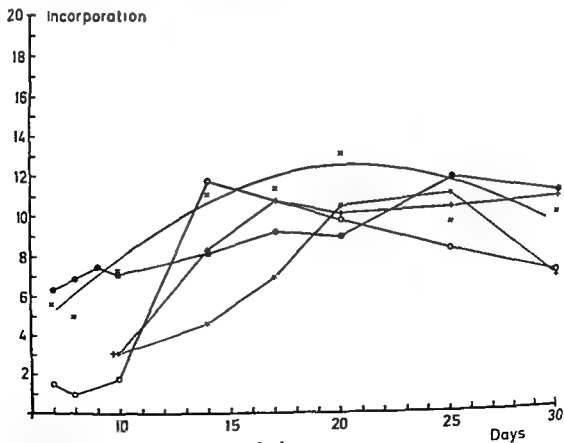
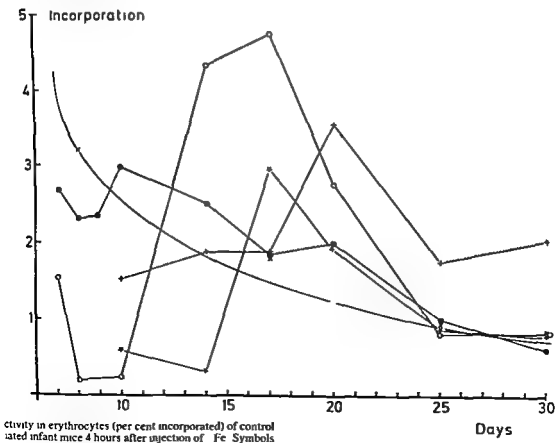
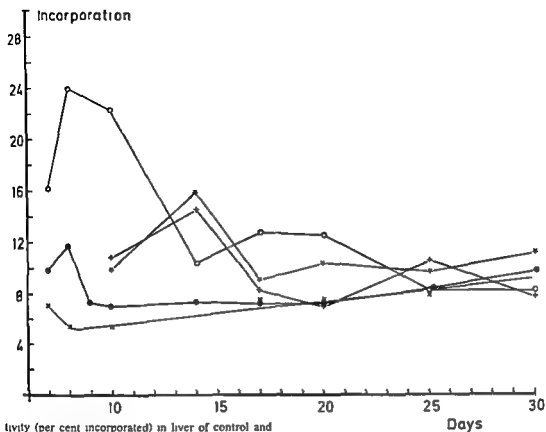


Fig 4 Activity (per cent incorporated) in spleen of control and irradiated infant mice 4 hours after injection of ^{59}Fe . Symbols as in Fig 1



ed changes in the spleen are less marked. These changes are in general somewhat more marked and last longer after a fractionated than after a single exposure to the same dose. An increase in iron incorporation occurring a few days after a dose of 1.26 Gy may be caused by a compensatory mechanism.

The lower turnover of serum iron during the first days after exposure reflect the lack of heme synthesizing cells in hemopoietic organs and allow a greater deposition of iron in the liver.

In conclusion, the observations confirm the higher effectiveness of a fractionated irradiation during infancy compared with a single one and indicate that these animals die from hemopoietic failure mainly due to an impaired maturation of the hemopoietic capacities of the bone marrow. It remains the task of future investigations to better define the cellular basis of these alterations.

SUMMARY

The erythropoiesis in control and irradiated infant mice was investigated on the basis of incorporation of iron into hemopoietic organs and blood. Iron incorporation in bone marrow increased from the second week of life reflecting the maturation of the marrow whereas that in the spleen showed only minor changes. Irradiation of infant mice on day 6 or 9 caused a bone marrow syndrome characterized by an impaired iron incorporation into hemopoietic tissues and blood by a reduced utilization of serum iron and particularly by a delayed maturation of the erythropoietic functions of bone marrow. Fractionated irradiation not

only had a greater effect on mortality but also caused more severe and long lasting depression of erythropoiesis than a single dose.

ACKNOWLEDGEMENTS

The investigation was supported by the Schutzkommission am Deutschen Innenministerium and contract No. II 232 76-1B10B of Biology Division of the Commission of the European Community (Publication No. 1708). The authors are grateful to Dr L. Feinendegen and Dr K. v. Wangenheim for valuable discussions.

REFERENCES

- BAUMANN B and MUTH H. Zur Frage der Erholungsfähigkeit von Säugetieren nach akuter sublethaler Ganzkörperbestrahlung mit energiereichen Strahlen unter Berücksichtigung des jugendlichen Organismus. In: Strahlenschutz in Forschung und Praxis, p. 35. Edited by O. Messerschmidt, G. Moehle and R. Zimmer. Georg Thieme Verlag, Stuttgart, 1977.
- BELCHER E H, GILBERT I G F and LAMERTON L F. Experimental studies with radioactive iron. *Brit J Radiol* 27 (1954) 387.
- BERAN M and TRIBUKAIT H. Changes in the natural resistance of the mouse after hypoxia of several days duration. The post hypoxic behaviour of stem cells. *Int J Radiat Oncol Biol Phys* 199 (1971) 27.
- GERBER G and ALTMAN K. I. Radiation biochemistry II, pp. 17-250. Edited by K. f. Altman, G. B. Gerber and S. Okada. Academic Press, New York, 1970.
- and MAES J. Stem cell kinetics in spleen and bone marrow after single and fractionated irradiation of infant mice. *Radiat Environm Biophys*. In press.
- METCALF H and MOORE M A S. Hemopoietic cells. North Holland Publ. Co., Amsterdam, 1971.

EFFECTS OF HYPERTHERMIA ON A NEUROGENIC RAT
CELL LINE (BT₄C) IN CULTURE

Development of thermal tolerance during continuous heating

OLAV DAHL

Hypoxic cells are at least as sensitive to hyperthermia as well oxygenated cells (GERWECK et coll 74 SCHULMAN & HALL 1974 HARISIADIS et coll 75 POWER & HARRIS 1977 GERWECK et coll 79). This may partly be due to an increased lactic acid production which lowers the pH under hypoxic conditions (OVERGAARD & SKOVGAARD POULSEN 1977 OVERGAARD & BICHEL 1977) or also lack of nutrients (HAHN 1974 OVERGAARD 1977). The probable existence of anoxic or hypoxic subpopulations due to necrosis and hemorrhage in gliomatous tumors (CHANG 1975) may contribute to the relatively poor effect of radiation therapy in these tumors.

The present investigation was initiated to examine the effects of supranormal temperatures (41–45°C) on the survival of malignant neurogenic cells in culture as a basis for experimental hyperthermia *in vivo*.

Materials and Methods

Cell line In all experiments a malignant neurogenic rat cell line designated BT₄C was used. The cell line originated after a single transplacental dose (75 µg/kg body weight) of N-ethyl-N-nitrosourea administered to pregnant female BD IX rats by intravenous injection on the 18th day of gestation (LÆRUM & RAJEWSKY 1975). The dissociated fetal brain cells grown in long term culture became

tumorigenic after about 200 days. The BT₄C line is composed of bi- or tripolar cells with a few giant cells and contains the specific nervous system protein S 100. When implanted subcutaneously in BD IX rats the cell line gives rise to pleomorphic or neurinoma like tumors (LÆRUM et coll 1977). The morphology was unaltered during the passages (45–70).

The BT₄C line has a doubling time of 18 to 20 hours when grown in asynchronous monolayer culture. The cells have triploid DNA content when analysed by flow cytometry (LÆRUM & HANSTEEN 1975).

Cell culture The cells were grown in Dulbecco's modification of Eagle's medium (DMEM Flow Laboratories Irvine) supplemented with 10% heat inactivated newborn calf serum, four fold concentration of non-essential amino acids, penicillin (100 IU/ml) and streptomycin (100 µg/ml). Monolayer cultures were maintained in 5% CO₂ in humidified air at 37°C. The cells were free of mycoplasma contamination when tested in agar under aerobic and anaerobic conditions.

For each experiment nearly confluent 3 day old monolayer cultures were rinsed once with phosphate buffered salt solution (PBS) and trypsinized (2

ed changes in the spleen are less marked. These changes are in general somewhat more marked and last longer after a fractionated than after a single exposure to the same dose. An increase in iron incorporation occurring a few days after a dose of 1.26 Gy may be caused by a compensatory mechanism.

The lower turnover of serum iron during the first days after exposure reflect the lack of heme synthesizing cells in hemopoietic organs and allow a greater deposition of iron in the liver.

In conclusion the observations confirm the higher effectiveness of a fractionated irradiation during in fancy compared with a single one and indicate that these animals die from hemopoietic failure mainly due to an impaired maturation of the hemopoietic capacities of the bone marrow. It remains the task of future investigations to better define the cellular basis of these alterations.

SUMMARY

The erythropoiesis in control and irradiated infant mice was investigated on the basis of incorporation of iron into hemopoietic organs and blood. Iron incorporation in bone marrow increased from the second week of life reflecting the maturation of the marrow whereas that in the spleen showed only minor changes. Irradiation of infant mice on day 6 or 9 caused a bone marrow syndrome characterized by an impaired iron incorporation into hemopoietic tissues and blood by a reduced utilization of serum iron and particularly by a delayed maturation of the erythropoietic functions of bone marrow. Fractionated irradiation not

only had a greater effect on mortality but also caused a more severe and long lasting depression of erythropoiesis than a single dose.

ACKNOWLEDGEMENTS

The investigation was supported by the Schutzkommission am Deutschen Innenministerium and contract No. B 232 76-IB10B of Biology Division of the Commission of the European Community (Publication No. 1708). The authors are grateful to Dr L. Feinendegen and Dr K. v. Wangenheim for valuable discussions.

REFERENCES

- BAUMANN H and MUTH H: Zur Frage der Erholungs-fähigkeit von Säugetieren nach akuter sublethaler Ganzkörperbestrahlung mit energiereichen Strahlen unter Berücksichtigung des jugendlichen Organismus. In: *Strahlenschutz in Forschung und Praxis* p. 35. Edited by O. Messerschmidt, G. Moehrle and R. Zimmer. Georg Thieme Verlag, Stuttgart, 1977.
- BELCHER H, GILBERT I G F and LAMERTON L F: Experimental studies with radioactive iron. *Brit J Radiol* 27 (1954) 387.
- BERAN M and TRIBUKAIT B: Changes in the natural resistance of the mouse after hypoxia of several days duration. The post hypoxic behaviour of stem cells. *Int J Radiat Oncol Biol Phys* 199 (1971) 77.
- GERBER G B and ALTMAN K I: Radiation biochemistry. II pp. 17-250. Edited by K. I. Altman, G. B. Gerber and S. Okada. Academic Press, New York, 1970.
- and MAES J: Stem cell kinetics in spleen and bone marrow after single and fractionated irradiation of infant mice. *Radiat Environm Biophys*. In press.
- METCALF D and MOORE M A S: Hemopoietic cells. North Holland Publ. Co., Amsterdam, 1971.

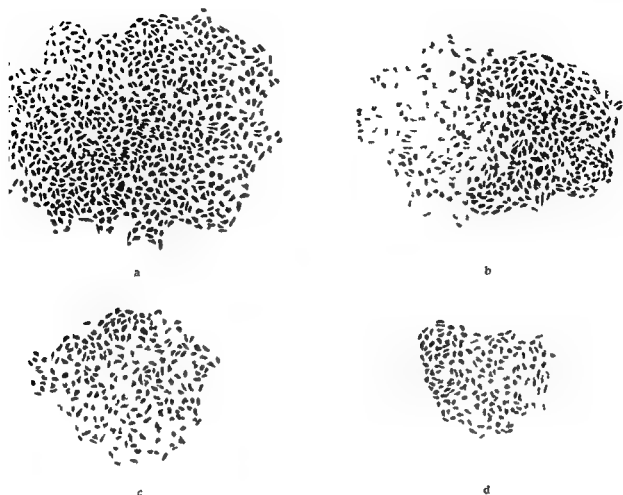


Fig. 1. Photomicrographs of stained colonies 8 days after exposure to 41.0°C for different times. a) Control 37.0°C b) 41.0°C for

2 h c) 41.0°C for 4 h d) 41.0°C for 12 h. Decrease in size after the longer incubation times. Magnification $\times 553$.

was in the water bath until they were removed. The temperature reached a range within 0.1°C of the red temperature in 4 to 5 min when heated from 37°C (data not shown).

Results

The survival curves have initial shoulders followed by logarithmic cell killing (Fig. 1). The linear part of the curves can be described by the parameter D_0 —the reciprocal of the slope of the curves—the duration in minutes at a particular temperature needed to reduce the survival to 1/e (37%) of the initial value (BHUYAN 1979). The size of the shoulder is expressed by the quasi threshold dose q —the time in minutes from start of therapy obtained by extrapolating the linear part of the curve surviving fraction = 1.0 (HARRIS 1977). The extrapolation number n is the intercept of the linear part of the curve and the y axis. The relation between these is

$$D_q = D_0 \times \ln n$$

The values for the three curve parameters at different temperatures are given in the Table.

No clonogenic cells were killed the first 8 hours at 40.0°C but after an incubation time of 12 hours a slight decrease occurred. At 41.0°C the linear part of the curve was biphasic with no further cell killing between 8 and 12 hours. The same biphasic response was indicated at about the same survival level at 42.0°C after 2 hours. This change was not caused by cell multiplication during treatment as proliferation during the hyperthermic treatment would give rise to greater colonies instead of more colonies. In fact a parallel decrease in colony size occurred with longer incubation times indicating a reduced proliferative capacity of the surviving cells (Figs 2, 3).

The reduction of the slope of the linear part of the curves at 41.0 and 42.0°C is probably due to

acquired resistance or thermal tolerance (defined as a decrease of the slope of the heat survival curve or an increase of D_0)

There is a great reduction of survival between 42.0 and 43.0°C. This is reflected in the Arrhenius plot (Fig. 4 JOHNSON et coll. 1954 WESTRA & DEWEY 1971 CONNOR et coll. 1977 BHUYAN) by a break of the curve at 43.0°C. The inflection indicates a change in activation energy μ which can be calculated from the Arrhenius equation

$$k = 1/D_0 = A \times e^{-\mu/RT} \text{ or } K_1 = K_0 \times e^{\frac{\mu}{R} \left(\frac{T_1 - T_0}{T_1 \times T_0} \right)}$$

where K is the slope of the survival curves ($1/D_0$) and A in the present temperature range (40–45°C) is practically a constant (BHUYAN). $A = K/h \times T \times e^{\Delta S/R}$ where K is Boltzmann's constant (1.38×10^{-16} erg/K) and h is Planck's constant (6.63×10^{-27} erg/s) and ΔS the change in inactivation entropy (BAUER & HENLE 1979). R is the gas constant 8.3 kJ/mol degree (2 kcal/mol degree) and T the absolute temperature in K. Inserting the values for $1/D_0$ (K_0 and K_1) at different temperatures (T_0 and T_1) in the latter equation then gives the activation energies for the cell line: between 41.0 and 43.0°C the activation energy is 1.175 kJ/mol (280 kcal/mol) and between 43.0 and 45.0°C the activation energy is 356 kJ/mol (85 kcal/mol) for heat killing of BT₄C cells.

Discussion

The survival of the neurogenic rat cell line BT₄C after exposure to elevated temperatures in culture is dependent on the temperature as well as the duration of the treatment as stated over 50 years ago both in vitro (LAMBERT 1912) and in animal experiments (WESTERMARK 1927).

Shoulders exist in the heat survival curves (Fig. 1) as found for most cell lines (BHUYAN). The contribution to the shoulders by the temperature equilibrium time (5 min) is negligible below 41.0°C but with the decrease of the shoulders at the higher temperatures the contribution of the equilibrium time also increases. Complex mechanisms like inherent cell resistance to heat or possible ability to accumulate and repair heat damage are also involved.

The heat survival curve parameters D_0 as an expression of the initial linear part of the curves pre-

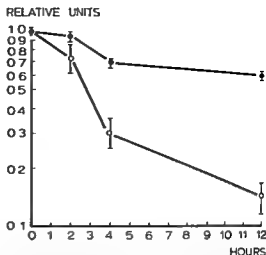


Fig. 3 Relative colony size as function of the incubation time at 41.0°C. Each point is the mean of 40 measured colonies after 8 days incubation at 37°C. ● = relative diameter of colonies (mean of controls 1.0). ○ = relative cell number (calculated by multiplying surviving fraction and colony area). Mean \pm SEM.

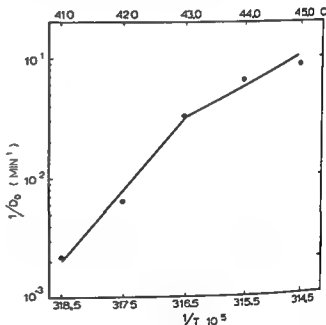


Fig. 4 Arrhenius plot for heat inactivation of BT₄C cells. The reciprocal values for D_0 are plotted versus the reciprocal of the absolute temperature (°K).

sented in the Table are in accordance with the results for several cell lines examined in vitro (ROBINSON & WIZENBERG 1974 BHUYAN). The BT₄C cells are more sensitive to heat than two human astrocytoma lines (GERWECK & BURLETT 1978) examined in vitro.

Colony size. Decrease in colony size as a function of the duration of hyperthermia (Figs 3) is re-

acquired resistance or thermal tolerance (defined as a decrease of the slope of the heat survival curve or an increase of D_0)

There is a great reduction of survival between 42.0 and 43.0 °C. This is reflected in the Arrhenius plot (Fig. 4 JOHNSON et coll 1954 WESTRA & DEWEY 1971 CONNOR et coll 1977 BHUYAN) by a break of the curve at 43.0 °C. The inflection indicates a change in activation energy μ which can be calculated from the Arrhenius equation

$$K = 1/D_0 = A \times e^{-\mu/RT} \text{ or } K_1 = K_0 \times e^{\frac{\mu}{R} \left(\frac{T_1 - T_0}{T_1 \times T_0} \right)}$$

where K is the slope of the survival curves ($1/D_0$) and A in the present temperature range (40–45 °C) is practically a constant (BHUYAN). $A = K/h \times T \times e^{\Delta S/R}$ where K is Boltzmann's constant (1.38×10^{-16} erg/K) and h is Planck's constant (6.63×10^{-3} erg/s) and ΔS the change in inactivation entropy (BAUER & HENLE 1979). R is the gas constant 8.3 kJ/mol degree (2 kcal/mol degree) and T the absolute temperature in K. Inserting the values for $1/D_0$ (K_0 and K_1) at different temperatures (T_0 and T_1) in the latter equation then gives the activation energies for the cell line: between 41.0 and 43.0 °C the activation energy is 1.175 kJ/mol (280 kcal/mol) and between 43.0 and 45.0 °C the activation energy is 356 kJ/mol (85 kcal/mol) for heat killing of BT₄C cells.

Discussion

The survival of the neurogenic rat cell line BT₄C after exposure to elevated temperatures in culture is dependent on the temperature as well as the duration of the treatment as stated over 50 years ago both in vitro (LAMBERT 1912) and in animal experiments (WESTERMARK 1927).

Shoulders exist in the heat survival curves (Fig. 1) as found for most cell lines (BHUYAN). The contribution to the shoulders by the temperature equilibrium time (5 min) is negligible below 41.0 °C but with the decrease of the shoulders at the higher temperatures the contribution of the equilibrium time also increases. Complex mechanisms like inherent cell resistance to heat or possible ability to accumulate and repair heat damage are also involved.

The heat survival curve parameters D_0 as an expression of the initial linear part of the curves pre-

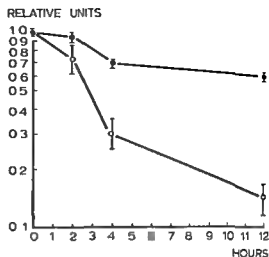


Fig. 3 Relative colony size as function of the incubation time at 41.0 °C. Each point is the mean of 40 measured colonies after days incubation at 37 °C. ● = relative diameter of colonies (from controls 1.0). ○ = relative cell number (calculated by multiplying surviving fraction and colony area). Mean \pm SEM.

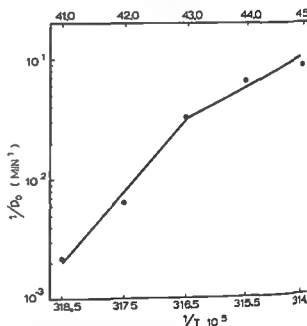


Fig. 4 Arrhenius plot for heat inactivation of BT₄C cells. Reciprocal values for D_0 are plotted versus the reciprocal of absolute temperature (K).

sented in the Table are in accordance with the results for several cell lines examined in vitro (ROBSON & WIZENBERG 1974 BHUYAN). The BT₄C cells are more sensitive to heat than two human astrocytoma lines (GERWECK & BURLETT 1978) examined in vitro.

Colony size. Decrease in colony size as a function of the duration of hyperthermia (Figs 2–3) is

curve (NIELSEN & OVERGAARD 1979) This type of thermal tolerance is found when both low temperatures (40–42°C MORGAN et coll 1979 JOSHI & JUNG 1979) and high temperatures (above 43°C GERNER & SCHNEIDER 1975 GERNER et coll 1976 HENLE & LEEPER 1976 HENLE et coll 1978 1979) precede the same or a higher temperature When the sequence is reversed and temperatures above 43 are followed by a lower temperature an enhanced cell killing is regularly found (GIOVANELLA et coll 1970 KANO et coll 1978 HENLE et coll 1979 JOSHI & JUNG MIYAKOSHI et coll 1979)

A mouse cell line (NIELSEN & OVERGAARD) which does not develop thermal tolerance during continuous heating clearly shows thermal tolerance by fractionated heating Whether these two types of thermal tolerance are caused by the same mechanism is not known

In vivo Thermal induced temporary resistance or tolerance to a second heat treatment *in vivo* was originally reported for mouse tumours (S91 melanoma and C₃Bl/6 sarcoma CRILE JR 1961 1963) as well as normal tissue (loss of foot) when preheated at 44°C one day before the second treatment

By using split dose hyperthermia (SUIT 1977) on normal tissue (loss of foot in mice) and on a fibrosarcoma minimum repair was found the first 5 hours after exposure to 43.5°C but the resistance increased at 22 hours interval When 10 fractions instead of one were used separated by 22 hours 5.3 and 4.4 times longer total exposure times were necessary to give the same result on normal tissue and tumours respectively

The effect of two separate heat treatments on normal tissue (necrosis of heated mouse ears) has recently been investigated (LAW et coll 1979a b) She had to increase the total heating time at 43.5°C in order to maintain the same level of injury if the interval between the fractions exceeded 4 hours The additional time increased until at 20 to 24 hours the second treatment was equal to the single dose time indicating full repair of the injury caused by the first treatment At longer intervals the second exposure had to be still longer reaching a maximum at 48 hours then a decay started with full extinction of the effect of the first dose after 96 hours She also found that the time of maximum thermal tolerance was dependent on the heat dose (time at 43.5°C)

Fractionated heating also induces resistance to

thermal enhancement of radiation injury *in vivo* (LAW et coll 1978 1979a)

Underlying mechanisms Thermal tolerance is defined as a concept describing the thermal induced change in slope of the heat survival curve i.e. the heat induced resistance to heating The term does not reveal the underlying mechanism although the conditions which promote or inhibit its development as well as the kinetics involved give some information Based on experiments several generalisations can be made The development of thermal tolerance takes time (2–20 hours GERWECK & ROTTINGER 1976 HARISIADIS et coll 1977 SAPARETO et coll GERNER et coll 1979 LAW et coll 1979b) reaching a maximum at 8 to 48 hours (HENLE et coll 1978 1979 LAW et coll 1979b NIELSEN & OVERGAARD then fading off in 1 to 2 days (HENLE & LEEPER LAW et coll 1979b NIELSEN & OVERGAARD) Thermal tolerance is not induced at 0°C (GERNER & SCHNEIDER) and it is inhibited by lowering pH (GERWECK NIELSEN & OVERGAARD) as well as in inhibiting the protein synthesis by cycloheximide (LEEPEER et coll 1977) These observations are some of the rationale of the hypothesis of an underlying metabolic process (GERNER & SCHNEIDER GERNER et coll 1976 HENLE et coll 1978 LEEPER et coll) possibly involving proteins or enzymes The cell membrane may also be involved in development of thermal tolerance (REEVES HENLE & LEEPER LI & HAHN 1978)

Thermal tolerance apparently does not depend on selection of resistant cell populations (HARISIADIS et coll 1975 GERNER et coll 1976) or a cell cycle redistribution (PALZER & HEIDELBERGER HARISIADIS et coll 1977 SAPARETO et coll BAUER & HENLE GERNER et coll 1979)

Thermal tolerance must be considered in planning animal and clinical hyperthermia Based on the knowledge that several tumours have lower pH than the surrounding normal tissue (for review see NIELSEN & OVERGAARD) and that there is a temperature gradient between tumours and surrounding tissue caused by vascular cooling (STORM et coll 1979) preferential induction of thermal tolerance in normal tissue may increase the therapeutic index (injury of tumours/injury of normal tissues) of hyperthermia

SUMMARY

The malignant neurogenic rat cell line BT C developed tolerance to further heat injury after continuous heating in

curve (NIELSEN & OVERGAARD 1979) This type of thermal tolerance is found when both low temperatures (40–42°C MORGAN et coll 1979 JOSHI & JUNG 1979) and high temperatures (above 43°C GERNER & SCHNEIDER 1975 GERNER et coll 1976 HENLE & LEEPER 1976 HENLE et coll 1978 1979) precede the same or a higher temperature When the sequence is reversed and temperatures above 43 are followed by a lower temperature an enhanced cell killing is regularly found (GIOVANELLA et coll 1970 KANO et coll 1978 HENLE et coll 1979 JOSHI & JUNG MIYAKOSHI et coll 1979)

A mouse cell line (NIELSEN & OVERGAARD) which does not develop thermal tolerance during continuous heating clearly shows thermal tolerance by fractionated heating Whether these two types of thermal tolerance are caused by the same mechanism is not known

In vivo Thermal induced temporary resistance or tolerance to a second heat treatment *in vivo* was originally reported for mouse tumours (S91 melanoma and C₂₇Bl/6 sarcoma CRILE JR 1961 1963) as well as normal tissue (loss of foot) when preheated at 44°C one day before the second treatment

By using split dose hyperthermia (SUIT 1977) on normal tissue (loss of foot in mice) and on a fibrosarcoma minimum repair was found the first 5 hours after exposure to 43.5°C but the resistance increased at 22 hours interval When 10 fractions instead of one were used separated by 22 hours 5.3 and 4.4 times longer total exposure times were necessary to give the same result on normal tissue and tumours respectively

The effect of two separate heat treatments on normal tissue (necrosis of heated mouse ears) has recently been investigated (LAW et coll 1979a, b) She had to increase the total heating time at 43.5°C in order to maintain the same level of injury if the interval between the fractions exceeded 4 hours The additional time increased until at 20 to 24 hours the second treatment was equal to the single dose time indicating full repair of the injury caused by the first treatment At longer intervals the second exposure had to be still longer reaching a maximum at 48 hours then a decay started with full extinction of the effect of the first dose after 96 hours She also found that the time of maximum thermal tolerance was dependent on the heat dose (time at 43.5°C)

Fractionated heating also induces resistance to

thermal enhancement of radiation injury *in vivo* (LAW et coll 1978 1979a)

Underlying mechanisms Thermal tolerance as defined is a concept describing the thermal induced change in slope of the heat survival curve i.e. the heat induced resistance to heating The term does not reveal the underlying mechanism although the conditions which promote or inhibit its development as well as the kinetics involved give some information Based on experiments several generalisations can be made The development of thermal tolerance takes time (2–20 hours GERWECK & ROTTINGER 1976 HARISADIS et coll 1977 SAPARETO et coll GERNER et coll 1979 LAW et coll 1979b) reaching a maximum at 8 to 48 hours (HENLE et coll 1978 1979 LAW et coll 1979b NIELSEN & OVERGAARD) then fading off in 1 to 2 days (HENLE & LEEPER LAW et coll 1979b NIELSEN & OVERGAARD) Thermal tolerance is not induced at 0°C (GERNER & SCHNEIDER) and it is inhibited by lowering pH (GERWECK NIELSEN & OVERGAARD) as well as in inhibiting the protein synthesis by cycloheximide (LEEPEL et coll 1977) These observations are some of the rationale of the hypothesis of an underlying metabolic process (GERNER & SCHNEIDER GERNER et coll 1976 HENLE et coll 1978 LEEPER et coll) possibly involving proteins or enzymes The cell membrane may also be involved in development of thermal tolerance (REEVES HENLE & LEEPER LI & HAHN 1978)

Thermal tolerance apparently does not depend on selection of resistant cell populations (HARISADIS et coll 1975 GERNER et coll 1976) or a cell cycle redistribution (PALZER & HEIDELBERGER HARISADIS et coll 1977 SAPARETO et coll BAUER & HENLE GERNER et coll 1979)

Thermal tolerance must be considered in planning animal and clinical hyperthermia Based on the knowledge that several tumours have lower pH than the surrounding normal tissue (for review see NIELSEN & OVERGAARD) and that there is a temperature gradient between tumours and surrounding tissue caused by vascular cooling (STORM et coll 1979) preferential induction of thermal tolerance in normal tissue may increase the therapeutic index (injury of tumours/injury of normal tissues) of hyperthermia

SUMMARY

The malignant neurogenic rat cell line BT/C developed tolerance to further heat injury after continuous heating in

- Radiation Essen 1977 Urban & Schwarzenberg Bal timore Munich 1978
- LERUM O D and HANSTEEN I L Chromosome analysis and cytofluorometric DNA measurements of malignant neurogenic cell lines in culture *In* Pulse cytophotometry p 172 Edited by C A M Haanen II F P Hillen and J M C Wessels European Press Medicom Ghent 1975
- and RAJEWSKY M F Neoplastic transformation of fetal rat brain cells in culture after exposure to ethylnitrosourea *in vivo* *J Nat Cancer Inst* 55 (1975) 1177
- SCHACHNER M STAVROU II HAGLID K G and HAUGEN Å Phenotypic properties of neoplastic cell lines developed from fetal rat brain cells in culture after exposure to ethylnitrosourea *in vivo* *Z Krebsforsch* 89 (1977) 273
- LAMBERT R A Demonstration of the greater susceptibility to heat of sarcoma cells as compared with actively proliferating connective tissue cells *J Amer med Ass* 54 (1912) 2147
- LANDRY J and MARCEAU N Cell growth recovery after treatments at various supraoptimal temperatures *Cancer Res* 39 (1979) 1218
- LAW M P AHIER R G and FIELDS II The response of the mouse ear to heat applied alone or combined with X rays *Brit J Radiol* 51 (1978) 132
- — — (a) The effect of prior heat treatment on the thermal enhancement of radiation damage in the mouse ear *Brit J Radiol* 52 (1979) 315
- COLLITAS P G and FIELD S B (b) Induced thermal resistance in the mouse ear *Brit J Radiol* 52 (1979) 308
- LEEIFR D B KARAMUZ J E and HENLE K J Effect of inhibition of macromolecular synthesis on the induction of thermotolerance *Proc Amer Ass Cancer Res* 18 (1977) 139
- LEITH J T MILLER R C GERNER E W and BOONE M L M Hyperthermic potentiation Biological aspects and applications to radiation therapy *Cancer* 39 (1977) 766
- LI G C and HAHN G M Ethanol induced tolerance to heat and to adriamycin *Nature* 274 (1978) 699
- MAGIN R L and JOHNSON R K Effects of local tumor hyperthermia on the growth of solid mouse tumors *Cancer Res* 39 (1979) 4534
- MİYAKOSHI J IKEBUCHI M FURUKAWA M YAMAGATA K SUGAHARA T and KANO E Combined effects of X irradiation and hyperthermia (42 and 44°C) on Chinese hamster V 79 cells *in vitro* *Radiat Res* 79 (1979) 77
- MORGAN J E HONESS II J and BLEEHEEN N M The interaction of thermal tolerance with drug cytotoxicity *in vitro* *Brit J Cancer* 39 (1979) 422
- MORRIS C C MYERS R and FIELD S B The response of the rat tail to hyperthermia *Brit J Radiol* 50 (1977) 576
- NIFLSEN O S and OVERGAARD J Effect of extracellular pH on thermotolerance and recovery of hyperthermic damage *in vitro* *Cancer Res* 39 (1979) 2772
- OVERGAARD J Effect of hyperthermia on malignant cells *in vivo* A review and a hypothesis *Cancer* 39 (1977) 2637
- and BICHEL P The influence of hypoxia and acidity on the hyperthermic response of malignant cells *in vitro* *Radiology* 123 (1977) 511
- and SKOVGAARD POULSEN H Effect of hyperthermia and environmental acidity on the proteolytic activity in murine ascites tumor cells *J Nat Cancer Inst* 58 (1977) 1159
- and SUIT H D Time-temperature relationship in hyperthermic treatment of malignant and normal tissue *in vivo* *Cancer Res* 39 (1979) 3248
- PALZER R J and HEIDELBERGER C Studies on the quantitative biology of hyperthermic killing of HeLa cells *Cancer Res* 33 (1973) 415
- POWER J A and HARRIS J W Response of extremely hypoxic cells to hyperthermia Survival and oxygen enhancement ratios *Radiology* 123 (1977) 767
- REFVES II O Mechanisms of acquired resistance to acute heat shock in cultured mammalian cells *J Cell Physiol* 79 (1972) 157
- ROBINSON J E and WIZENBERG M J Thermal sensitivity and the effect of elevated temperatures on the radiation sensitivity of Chinese hamster cells *Acta radiol Ther Phys Biol* 13 (1974) 241
- SAPARETOS A HOPWOOD L E DEWEY W C RAU M R and GRAY J W Effects of hyperthermia on survival and progression of Chinese hamster ovary cells *Cancer Res* 38 (1978) 393
- SCHULMAN N and HALL E J Hyperthermia Its effect on proliferative and plateau phase-cell cultures *Radiology* 113 (1974) 209
- STORM F K HARRISON W H ELLIOTT R S and MORTON D L Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials *Cancer Res* 39 (1979) 2245
- SUIT H II Hyperthermic effects on animal tissues *Radiology* 123 (1977) 483
- WESTERMARK N The effect of heat upon rat tumors *Skand Arch Physiol* 52 (1927) 257
- WESTRA A and DEWEY W C Variation in sensitivity to heat shock during the cell cycle of Chinese hamster cells *in vitro* *Int J Radiat Biol* 19 (1971) 467

- Radiation Essen 1977 Urban & Schwarzenberg, Bal timore Munich 1978
- LEFRUM O D and HANSTEEN I L Chromosome analysis and cytofluorometric DNA measurements of malignant neurogenic cell lines in culture *In*: Pulse cytophotometry p 172 Edited by C A M Haanen H F P Hillen and J M C Wessels European Press Medicon Ghent 1975
- and RAJEWSKY M F Neoplastic transformation of fetal rat brain cells in culture after exposure to ethylnitrosourea in vivo *J Nat Cancer Inst* 55 (1975) 1177
- — SCHACHNER M STAVROU D HAGLID K G and HAUGEN A Phenotypic properties of neoplastic cell lines developed from fetal rat brain cells in culture after exposure to ethylnitrosourea in vivo *Z Krebsforsch* 89 (1977) 273
- LANIBFTR A Demonstration of the greater susceptibility to heat of sarcoma cells as compared with actively proliferating connective tissue cells *J Amer med Ass* 54 (1912) 2147
- LANDRY J and MARCEAU N Cell growth recovery after treatments at various supraoptimal temperatures *Cancer Res* 39 (1979) 1218
- LAW M P AHIER R G and FIELD S B The response of the mouse ear to heat applied alone or combined with X rays *Brit J Radiol* 51 (1978) 132
- — — (a) The effect of prior heat treatment on the thermal enhancement of radiation damage in the mouse ear *Brit J Radiol* 52 (1979) 315
- COULTAS P G and FIELD S B (b) Induced thermal resistance in the mouse ear *Brit J Radiol* 52 (1979) 308
- LEEPER D B KARAMUZ J E and HFALE K J Effect of inhibition of macromolecular synthesis on the induction of thermotolerance *Proc Amer Ass Cancer Res* 18 (1977) 139
- LEITH J T MILLER R C GERNER E W and BOONE M L M Hyperthermic potentiation: Biological aspects and applications to radiation therapy *Cancer* 39 (1977) 766
- LIG C and HAHN G M Ethanol induced tolerance to heat and to adriamycin *Nature* 274 (1978) 699
- MAGINR L and JOHNSON R K Effects of local tumor hyperthermia on the growth of solid mouse tumors *Cancer Res* 39 (1979) 4534
- MIYAKOSHI J IKBUCHI M FURUKAWA M YAMAGATA K SUGAHARA T and KANO E Combined effects of X irradiation and hyperthermia (42 and 44°C) on Chinese hamster V 79 cells in vitro *Radiat Res* 79 (1979) 77
- MORGAN J E HONESS D J and BLEEHEEN N M The interaction of thermal tolerance with drug cytotoxicity in vitro *Brit J Cancer* 39 (1979) 422
- MORRIS C C MYERS R and FIELD S B The response of the rat tail to hyperthermia *Brit J Radiol* 50 (1977) 576
- NIELSEN O S and OVERGAARD J Effect of extracellular pH on thermotolerance and recovery of hyperthermic damage in vitro *Cancer Res* 39 (1979) 2772
- OVERGAARD J Effect of hyperthermia on malignant cells in vivo: A review and a hypothesis *Cancer* 39 (1977) 2637
- and BICHEP P The influence of hypoxia and acidity on the hyperthermic response of malignant cells in vitro *Radiology* 123 (1977) 511
- and SKOVGAARD POULSEN H Effect of hyperthermia and environmental acidity on the proteolytic activity in murine ascites tumor cells *J Nat Cancer Inst* 58 (1977) 1159
- and SUIT H D Time-temperature relationship in hyperthermic treatment of malignant and normal tissue in vivo *Cancer Res* 39 (1979) 3248
- PALZER R J and HEIDELBERGER C Studies on the quantitative biology of hyperthermic killing of HeLa cells *Cancer Res* 33 (1973) 415
- POWER J A and HARRIS J W Response of extremely hypoxic cells to hyperthermia: Survival and oxygen enhancement ratios *Radiology* 123 (1977) 767
- REEVES R O Mechanisms of acquired resistance to acute heat shock in cultured mammalian cells *J Cell Physiol* 79 (1972) 157
- ROBINSON J E and WIZFENBERG M J Thermal sensitivity and the effect of elevated temperatures on the radiation sensitivity of Chinese hamster cells *Acta radiol Ther Phys Biol* 13 (1974) 241
- SAPARETO S A HOPWOOD L E DEWEY W C RAU M R and GRAY J W Effects of hyperthermia on survival and progression of Chinese hamster ovary cells *Cancer Res* 38 (1978) 393
- SCHULMAN N and HALL E J Hyperthermia: Its effect on proliferative and plateau phase cell cultures *Radiology* 113 (1974) 209
- STORM F K HARRISON W H ELLIOTT R S and MORTON D L Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials *Cancer Res* 39 (1979) 2245
- SUIT H D Hyperthermic effects on animal tissues *Radiology* 123 (1977) 483
- WESTERNARK N The effect of heat upon mit tumors *Skand Arch Physiol* 52 (1927) 257
- WESTRA A and DEWEY W C Variation in sensitivity to heat shock during the cell cycle of Chinese hamster cells in vitro *Int J Radiat Biol* 19 (1971) 467

- Radiation Essen 1977 Urban & Schwarzenberg Baltimore Munich 1978
- LERUM O D and HANSTEEN I L Chromosome analysis and cytofluorometric DNA measurements of malignant neurogenic cell lines in culture *In Pulse cytophotometry* p 172 Edited by C A M Haanen H F P Hillen and J M C Wessels European Press Medicon Ghent 1975
- and RAJEWSKY M F Neoplastic transformation of fetal rat brain cells in culture after exposure to ethylnitrosourea in vivo *J nat Cancer Inst* 55 (1975) 1177
- SCHACHNER M STAVROU D HAGLID K G and HAUGEN Å Phenotypic properties of neoplastic cell lines developed from fetal rat brain cells in culture after exposure to ethylnitrosourea in vivo *Z Krebsforsch* 89 (1977) 273
- LAMBERT R A Demonstration of the greater susceptibility to heat of sarcoma cells as compared with actively proliferating connective tissue cells *J Amer med Ass* 54 (1912) 2147
- LANDRY J and MARCEAU N Cell growth recovery after treatments at various supraoptimal temperatures *Cancer Res* 39 (1979) 1218
- LAW M P AHIER R G and FIELD S B The response of the mouse ear to heat applied alone or combined with X rays *Brit J Radiol* 51 (1978) 132
- — (a) The effect of prior heat treatment on the thermal enhancement of radiation damage in the mouse ear *Brit J Radiol* 52 (1979) 315
- COULTAS P G and FIELD S B (b) Induced thermal resistance in the mouse ear *Brit J Radiol* 52 (1979) 308
- LEEPER D M KARAMUZ J E and HENLE K J Effect of inhibition of macromolecular synthesis on the induction of thermotolerance *Proc Amer Ass Cancer Res* 18 (1977) 139
- LEITH J T MILLER R C GERNER E W and BOONE M L M Hyperthermic potentiation Biological aspects and applications to radiation therapy *Cancer* 39 (1977) 766
- LI G C and HAHN G M Ethanol induced tolerance to heat and to adriamycin *Nature* 274 (1978) 699
- MAGIN R L and JOHNSON R K Effects of local tumor hyperthermia on the growth of solid mouse tumors *Cancer Res* 39 (1979) 4534
- MIYAKOSHI J IKEBUCHI M FURUKAWA M YAMAGATA K SUGAHARA T and KANO E Combined effects of X irradiation and hyperthermia (42 and 44°C) on Chinese hamster V 79 cells in vitro *Radiat Res* 79 (1979) 77
- MORGAN J E HONESS D J and BLEEHEEN N M The interaction of thermal tolerance with drug cytotoxicity in vitro *Brit J Cancer* 39 (1979) 422
- MORRIS C C MYERS R and FIELD S B The response of the rat tail to hyperthermia *Brit J Radiol* 50 (1977) 576
- NIELSEN O S and OVERGAARD J Effect of extracellular pH on thermotolerance and recovery of hyperthermic damage in vitro *Cancer Res* 39 (1979) 2772
- OVERGAARD J Effect of hyperthermia on malignant cells in vivo A review and a hypothesis *Cancer* 39 (1977) 2637
- and BICHEL P The influence of hypoxia and acidity on the hyperthermic response of malignant cells in vitro *Radiology* 123 (1977) 511
- and SKOVGAARD POULSEN H Effect of hyperthermia and environmental acidity on the proteolytic activity in murine ascites tumor cells *J Nat Cancer Inst* 58 (1977) 1159
- and SUIT H D Time-temperature relationship in hyperthermic treatment of malignant and normal tissue in vivo *Cancer Res* 39 (1979) 3248
- PALZER R J and HEIDELBERGER C Studies on the quantitative biology of hyperthermic killing of HeLa cells *Cancer Res* 33 (1973) 415
- POWER J A and HARRIS J W Response of extremely hypoxic cells to hyperthermia Survival and oxygen enhancement ratios *Radiology* 123 (1977) 767
- REEVES R O Mechanisms of acquired resistance to acute heat shock in cultured mammalian cells *J Cell Physiol* 79 (1972) 157
- ROBINSON J E and WIZENBERG M J Thermal sensitivity and the effect of elevated temperatures on the radiation sensitivity of Chinese hamster cells *Acta radiol Ther Phys Biol* 13 (1974) 241
- SAPARETOS A HOPWOOD L E DEWEY W C RAU M R and GRAY J W Effects of hyperthermia on survival and progression of Chinese hamster ovary cells *Cancer Res* 38 (1978) 393
- SCHULMAN N and HALL E J Hyperthermia Its effect on proliferative and plateau phase-cell cultures *Radiology* 113 (1974) 209
- STORK F K HARRISON W H ELLIOTT R B and MORTON D L Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials *Cancer Res* 39 (1979) 2245
- SUIT H D Hyperthermic effects on animal tissues *Radiology* 123 (1977) 483
- WESTERMARK N The effect of heat upon rat tumors *Skand Arch Physiol* 52 (1927) 257
- WESTRA A and DEWEY W C Variation in sensitivity to heat shock during the cell cycle of Chinese hamster cells in vitro *Int J Radiat Biol* 19 (1971) 467

- Radiation Essen 1977 Urban & Schwarzenberg Bal timore Munich 1978
- L FRUM O D and HANSTEEN I L Chromosome analysis and cytofluorometric DNA measurements of malignant neurogenic cell lines in culture *In* Pulse cytophotometry p 172 Edited by C A M Haanen H F P Hillen and J M C Wessels European Press Medicon Ghent 1975
- and RAJCEWSKY M F Neoplastic transformation of fetal rat brain cells in culture after exposure to ethylnitrosourea *in vivo* *J nat Cancer Inst* 55 (1975) 1177
- — — SCHACHNER M STAVROU D HADLID K G and HAUGEN A Phenotypic properties of neoplastic cell lines developed from fetal rat brain cells in culture after exposure to ethylnitrosourea *in vivo* *Z Krebsforsch* 89 (1977) 273
- LAMBERT R A Demonstration of the greater susceptibility to heat of sarcoma cells as compared with actively proliferating connective tissue cells *J Amer med Ass* 54 (1912) 2147
- LANDRY J and MARCEAU N Cell growth recovery after treatments at various supraoptimal temperatures *Cancer Res* 39 (1979) 1218
- LAW M P AHIER R G and FIELDS S B The response of the mouse ear to heat applied alone or combined with X rays *Brit J Radiol* 51 (1978) 132
- — — (a) The effect of prior heat treatment on the thermal enhancement of radiation damage in the mouse ear *Brit J Radiol* 52 (1979) 315
- — — COLTAS P G and FIELDS S B (b) Induced thermal resistance in the mouse ear *Brit J Radiol* 52 (1979) 308
- LEPPER D B KARAMLIZI E and HENLE K J Effect of inhibition of macromolecular synthesis on the induction of thermotolerance *Proc Amer Ass Cancer Res* 18 (1977) 139
- LEITH J T MILLER R C GERNER E W and BOONE M L M Hyperthermic potentiation Biological aspects and applications to radiation therapy *Cancer* 39 (1977) 766
- LIG C and HAHN G M Ethanol induced tolerance to heat and to adriamycin *Nature* 274 (1978) 699
- MAGINR L and JOHNSON R K Effects of local tumor hyperthermia on the growth of solid mouse tumors *Cancer Res* 39 (1979) 4534
- MIYAKOSHI J IKEBUCHI M FURUKAWA M YAMAGATA K SUGAHARA T and KANO E Combined effects of X irradiation and hyperthermia (42 and 44°C) on Chinese hamster V 79 cells *in vitro* *Radiat Res* 79 (1979) 77
- MORGAN J E HONESS D J and BLEEHEEN N M The interaction of thermal tolerance with drug cytotoxicity *in vitro* *Brit J Cancer* 39 (1979) 422
- MORRIS C C MYERS R and FIELD S B The response of the rat tail to hyperthermia *Brit J Radiol* 50 (1977) 576
- NIELSEN O S and OVERGAARD J Effect of extracellular pH on thermotolerance and recovery of hyperthermic damage *in vitro* *Cancer Res* 39 (1979) 2772
- OVERGAARD J Effect of hyperthermia on malignant cells *in vivo* A review and a hypothesis *Cancer* 39 (1977) 2637
- and BICHEL P The influence of hypoxia and acidity on the hyperthermic response of malignant cells *in vitro* *Radiology* 123 (1977) 511
- and SKOVGAARD POULSEN H Effect of hyperthermia and environmental acidity on the proteolytic activity in murine ascites tumor cells *J Nat Cancer Inst* 58 (1977) 1159
- and SUIT H D Time-temperature relationship in hyperthermic treatment of malignant and normal tissue *in vivo* *Cancer Res* 39 (1979) 3248
- PALZER R J and HEIDELBERGER C Studies on the quantitative biology of hyperthermic killing of HeLa cells *Cancer Res* 33 (1973) 415
- POWER J A and HARRIS J W Response of extremely hypoxic cells to hyperthermia Survival and oxygen enhancement ratios *Radiology* 123 (1977) 767
- REEVES R O Mechanisms of acquired resistance to acute heat shock in cultured mammalian cells *J Cell Physiol* 79 (1972) 157
- ROBINSON J E and WIZENBERG M J Thermal sensitivity and the effect of elevated temperatures on the radiation sensitivity of Chinese hamster cells *Acta radiol Ther Phys Biol* 13 (1974) 241
- SAPARETO S A HOPWOOD L E DEWEY W C RAUM R and GRAY J W Effects of hyperthermia on survival and progression of Chinese hamster ovary cells *Cancer Res* 38 (1978) 393
- SCHULMAN N and HALL E J Hyperthermia Its effect on proliferative and plateau phase cell cultures *Radiology* 113 (1974) 209
- STORM F K HARRISON W H ELLIOTT R S and MORTON D L Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials *Cancer Res* 39 (1979) 2245
- SUIT H D Hyperthermic effects on animal tissues *Radiology* 123 (1977) 483
- WESTERMARK N The effect of heat upon rat tumors *Skand Arch Physiol* 52 (1927) 257
- WESTRA A and DEWEY W C Variation in sensitivity to heat shock during the cell cycle of Chinese hamster cells *in vitro* *Int J Radiat Biol* 19 (1971) 467

ACTA RADIOLOGICA

FOUNDED IN 1921 BY GÖSTA FOKSSJÖ

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK, FINLAND, NORWAY AND SWEDEN

ONCOLOGY

RADIATION THERAPY PHYSICS AND BIOLOGY

EDITOR

ERIK LINDGREN

ADVISORY BOARD

Oncology Lars Gunnar Larsson

Radiation physics Hans Svensson

Radiation biology Bernhard Tribukait

ASSOCIATE EDITORS

Ulf Rudén Ulf Hjärvald

EDITORIAL BOARD

Denmark G. Thomsen & Knud

Finland P. Virtanen & H. Holsti

Norway J. Ankhus & P. Poppe

Sweden E. G. Larsson & P. Saltzman

INDICES

Volume 19 (1980)

CONTENTS OF VOLUME 19—ONCOLOGY

Editorial	1	Significance of quantum fluctuations in roentgen imaging	
Laryngeal carcinoma—IV—Analysis of treatment results using the Cohen model		K-G STRID	129
M HJELM HANSEN	3	Natural killer activity in peripheral lymphocyte population following radiation therapy	
Complications from irradiation of carcinoma of the uterine cervix		II BLOMGREN E BARAL F EDSMYR L E	139
J ALERT J JIMENEZ L BELDARRAIN J MONT ALVO and C ROCA	13	Effect of different ^{90}Sr doses on the microscopic structure of foetal mouse ovaries	
Chromosome counts of ^{90}Sr induced osteosarcomas in mice—I—Transplanted tumour series		C RÖNNBACK	145
II BERGMAN and A NILSSON	17	Chromosome counts of ^{90}Sr induced osteosarcomas in mice—II—Variation of the chromosome counts of slow and fast growing tumours in hyper and non hyperimmunized hosts	
Effect of syngeneic bone marrow and thymus cell transplantation to ^{90}Sr irradiated mice	29	H BERGMAN	153
A NILSSON P BIERKE and A BROOMÉ KARLSSON	37	Granulosa and theca-cell tumors—Incidence and occurrence of second primary tumors	
Simple experimentally derived algorithm for computer calculated dose rates associated with ^{137}Cs gynecologic implants		III BJÖRKHOLM and C SILFVERSWARD	161
D E WREDE and H DAWALIBI	41	Prolactin secreting pituitary adenoma—Observations in irradiated patients	
Computation of dose distributions for radioactive seed implants		A DE SCHRYVER D VANDEKERCKHOVE and G DEBRUYNE	169
I I ROSEN R G LANE and C A KELSEY	45	Primary mucosal malignant melanoma—Appraisal of role of radiation therapy	
Effects of washing on phytohemagglutinin responsiveness of lymphocytes from irradiated patients		Y H SON	177
S MATSUBARA J HORIUCHI H SHIBUYA and M S SASAKI	55	Hodgkin's disease clinical stages I and II—Results of radical irradiation with or without chemotherapy	
Procedures in External Radiation Therapy Dosimetry with Electron and Photon Beams with Maximum Energies between 1 and 50 MeV—Recommendations by the Nordic Association of Clinical Physics (NACP)		B HERNI H EGHBALI M DURAND A DE MASCAREL G HERNI SIMON P RICHAUD J CHAUVERGNE and C LAGARDE	183
Mitomycin C in advanced gallbladder carcinoma	81	Chloramphenicol toxicity in radiation disease	
F VON EYBEN C HELLEKANT W MATSSON U LUNGUIST and K JONSSON		M POSPIŠIL L BENES L TKADLEČEK J VÁCHA V VELČOVSKÝ J NETIKOVA and Š VIKLICKÁ	193
Pentagastrin calcium and whisky stimulated serum calcitonin in medullary carcinoma of the thyroid		Collimation of high energy electron beams	
K EMVERTSEN H E NIELSEN L MOSEKILDE and H HVID HANSEN	85	I LAY and A BRAHME	199
Neutron radiation therapy of parotid gland tumors		Microdosimetry—II—Use of secondary electron emission to simulate two target models	
J P GERACI	91	T E BURLIN and B J FORSBERG	209
Effect of different radiation fractionation schedules on metastases from an oesophageal carcinoma		Chromosome counts of ^{90}Sr -induced osteosarcomas in mice—III—Variation of the chromosome counts of in vivo transplanted tumours in vitro cultures and retransplanted cultured cells	
C MERCKE I L LAMM P NILSSON T LAND BERG C H HAKANSSON and E HAMMAR	99	H BERGMAN	215
Depth dose data for 4 MeV linear accelerators with lead or uranium field flatteners		Age and dose related carcinogenicity of ^{90}Sr	
R NAIR and D E WREDE	107	A NILSSON P BIERKE G WALINDER and A BROOMÉ KARLSSON	223
Dual photon absorptiometry in lumbar vertebrae—Evaluation of the baseline error		Dose distribution around radium arrays used in the treatment of uterine carcinoma	
B O ROOS T H HANSSON and H SKÖLDBORN	111	F D SHARMA and J C F MACDONALD	229
Microdosimetry—I—Use of secondary electron emission		Irradiation injury of bone tissue—A vital microscopic method	
B J FORSBERG and T R BURLIN	115	T ALBREKTSSON M JACOBSSON and I TURESSON	235

Theca cell tumors—Clinical features and prognosis E BJÖRKHOLM and C SILFVERSWÄRD	241	Effects of acute gamma irradiation on spermatogenesis as revealed by flow cytometry U HACKER J SCHUMANN and W GÖDHE	361
Sensitivity of cells in exponential and stationary growth phase to combined treatment with radiation and Quinacrine J MIDANDER and B LITTEBRAND	245	Uptake of serotonin liberated by irradiation of rabbits and mice T VENINGA and W LEMSTRA	369
Calcitonin and mammary carcinoma L WAHLBY and G WESTMAN	251	Induction of pituitary tumours by combination of oestrogenic hormones and ^{90}Sr A NILSSON P BIERKE L HARALDSSON and A BROOMÉ KARLSSON	373
Effect of a screening programme on breast carcinoma incidence mortality and survival I SOINI and M HAKAMA	255	Effects of proton irradiation of the lumbar intumescent on intra axonal transport of acetylcholine and cholinergic enzymes in rat sciatic nerve S BÖÖJ A DAHLSTRÖM P A LARSSON K ROSANDER and B ROSENGREN	387
Effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma in athymic nude mice E K ROFSTAD T LINDMO and T BRUSTAD	261	Irradiation combined with Bleomycin treatment of synchronized cells in culture under oxic and hypoxic conditions J MIDANDER B LITTEBRAND and F EDSMYR	395
Dose effect relationships in cervical and thoracic radiation myelopathies B HOLDORFF	271	Nuclear imaging of pulmonary metastases in thyroid carcinoma A M WORM I HOLTEN and E TAANING	401
Chromosome counts of ^{90}Sr induced osteosarcomas in mice—IV—Variation of chromosome counts when using tumours of predetermined age for transplantation H BERGMAN	279	Microprocessor system for tracking isodensity lines in film dosimetry R VAN DER LAARSE and J DE GANS	405
Radiation sensitivity of DNA molecules in situ in normal and neoplastic tissues of mice T ONO K SAKAMOTO and S OKADA	285	Photon energy spectra of a heavily filtered 300 kV therapy unit T S CHEN K R KASE and B E BJARNGARD	411
Induction of neoplasia by ^{140}Ba in mice A NILSSON P BIERKE and A BROOMÉ KARLSSON	293	Superfractionated irradiation combined with low doses of Bleomycin in the treatment of oral carcinoma C LINDHOLM B LITTEBRAND and P O LÖFROTH	417
Influence of thyroid hormones on erythropoiesis and radiation resistance in C 57 black mice J MIKESKA M POSPIŠIL J NETIKOVÁ and B HOŠEK	299	Radiation therapy of glottic carcinoma stage I B Jose D L CALHOUN A MOHAMMED and D A TOBIN	471
Electron and photon beams from a 50 MeV racetrack microtron A BRAHME T KRAEPELIEN and H SVENSSON	305	Advanced carcinoma of the tonsil—Treatment results Z PETROVICH H KUISK L JOSE B BARTON and D RICE	475
Short term intra arterial Mitomycin C in hepatic metastases W MATSSON K JONSSON C HELLEKANT and L HALLSTEN	321	Radiation therapy of nasopharyngeal carcinoma C CHANG T LIU Y CHANG and S CAO	433
^{32}P pyrophosphate in the treatment of persistent metastatic bone pain B WERNER C ISACSON G LUNDELL C LÖNN BECK and B SÖDERBORG	327	Fast neutron teletherapy for advanced carcinoma of hypopharynx and supraglottic larynx G E LARAMORE J JOHNSON T W GRIFFIN D TONG M T GROUNDINE J M KURTZ A H RUSSELL and R G PARKER	439
Serum calcitonin in patients with osteolytic and osteosclerotic metastases from mammary carcinoma H E NIELSEN and C CH GADEBERG	331	Hypothyroidism following ^{131}I therapy for hyperthyroidism in relation to immunologic parameters G LUNDELL and L E HOLM	449
Palliative irradiation of brain metastases C I C TURALBA A M EL MAHDI and W J PEEPLES	335	Thyroid treatment and its possible influence on occurrence of malignant tumors after diagnostic ^{131}I L E HOLM	455
Transient intestinal ischaemia induced by degradable starch microspheres—Experiments in the cat K LÖTE M FÖLLING B ROSENGREN K SVANES and J LEKVEN	343	Bone abnormalities in patients with medullary carcinoma of the thyroid B RASMUSSEN	461
Radiation therapy of primary vaginal carcinoma L PIRTOLI and R SANTONI	353	Invasive squamous cell carcinoma of the uterine cervix—I—Definition of parameters in a histopathologic malignancy grading system U STENDAHL H WILLEN and R WILLEN	467
Relationship between histologic grading of head and neck tumours and regression after chemotherapy K JØRGENSEN and J SCHLICHTING	357		

Survival in carcinoma of the uterine cervix correlated to primary treatment results J JIMENEZ J ALERT L BALDARRAIN J MONT ALVO and C ROCA	481	Effects of hyperthermia on a neurogenic rat cell line (BT ₄ C) in culture—Development of thermal toler- ance during continuous heating O DAHL	489
Iron incorporation after single and fractionated ir- radiation of infant mice G B GERBER and J MAES	483		

SUBJECT INDEX TO VOLUME 19—ONCOLOGY, RADIATION THERAPY PHYSICS AND BIOLOGY

Oncology

Granulosa and theca-cell tumors—Incidence and occurrence of second primary tumors	161
Prolactin secreting pituitary adenoma irradiated patients	169
Theca cell tumors—Clinical features and prognosis	241
Calcitonin and mammary carcinoma	251
Effect of screening programme on breast carcinoma incidence mortality and survival	255
Serum calcitonin in patients with osteolytic and osteosclerotic metastases from mammary carcinoma	331
Relationship between histologic grading of head and neck tumours and regression after chemotherapy	357
Hypothyroidism following ^{131}I therapy for hyperthyroidism in relation to immunologic parameters	449
Bone abnormalities in patients with medullary carcinoma of the thyroid	461
Invasive squamous cell carcinoma of the uterine cervix—I—Definition of parameters in a histopathologic malignancy grading system	467
Survival in carcinoma of the uterine cervix correlated to primary treatment results	481

Radiation therapy

Laryngeal carcinoma—IV—Analysis of treatment results using the Cohen model	3
Complications from irradiation of carcinoma of the uterine cervix	13
Effects of washing on phytohemagglutinin responsiveness of lymphocytes from irradiated patients	45
Pentagastrin calcium and whisky stimulated serum calcitonin in medullary carcinoma of the thyroid	85
Neutron radiation therapy of parotid gland tumors	91
Effect of different radiation fractionation schedules on metastases from an oesophageal carcinoma	99
Natural killer activity in peripheral lymphocyte population following radiation therapy	139
Prolactin secreting pituitary adenoma irradiated patients	169
Primary mucosal malignant melanoma—Role of radiation therapy	177
Hodgkin's disease clinical stages I and II—Radical irradiation with or without chemotherapy	183
Dose distribution around radium arrays used in the treatment of uterine carcinoma	229
Calcitonin and mammary carcinoma	251
Dose effect relationships in radiation myelopathies	271
^{32}P pyrophosphate in the treatment of persistent metastatic bone pain	327

Serum calcitonin in patients with osteolytic and osteosclerotic metastases from mammary carcinoma	331
Palliative irradiation of brain metastases	335
Radiation therapy of primary vaginal carcinoma	353
Effects of acute gamma irradiation on spermatogenesis as revealed by flow cytometry	361
Superfractionated irradiation combined with Bleomycin in the treatment of oral carcinoma	417
Radiation therapy of glottic carcinoma stage I	471
Advanced carcinoma of the tonsil—Treatment results	425
Radiation therapy of nasopharyngeal carcinoma	433
Fast neutron teletherapy for advanced carcinoma of hypopharynx and supraglottic larynx	439
Hypothyroidism following ^{131}I therapy for hyperthyroidism in relation to immunologic parameters	449
Thyroid treatment and its possible influence on occurrence of malignant tumors after diagnostic ^{131}I	455
Survival in carcinoma of the uterine cervix correlated to primary treatment results	481

Hormone or Chemotherapy

Mitomycin C in advanced gallbladder carcinoma	81
Hodgkin's disease clinical stages I and II—Radical irradiation with or without chemotherapy	183
Short term intra arterial Mitomycin C in hepatic metastases	371
Relationship between histologic grading of head and neck tumours and regression after chemotherapy	357
Superfractionated irradiation combined with Bleomycin in the treatment of oral carcinoma	417

Isotopes

Chromosome counts of ^{90}Sr induced osteosarcomas in mice—I—Transplanted tumour series	17
Effect of syngeneic bone marrow and thymus cell transplantation to ^{90}Sr irradiated mice	29
Simple experimentally derived algorithm for computer calculated dose rates associated with ^{137}Cs gynecologic insertions	37
Computation of dose distributions for radioactive seed implants	41
Effects of washing on phytohemagglutinin responsiveness of lymphocytes from irradiated patients	45
Effect of different ^{90}Sr doses on the microscopic structure of foetal mouse ovaries	145
Chromosome counts of ^{90}Sr induced osteosarcomas in mice—II—Slow and fast growing tumours in hyper and non hyperimmunized hosts	153

- Chromosome counts of ^{90}Sr induced osteosarcomas in mice—III—In vivo transplanted tumours in vitro cultures and retransplanted cultured cells 215
- Age and dose related carcinogenicity of ^{90}Sr 223
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice—IV—When using tumours of predetermined age for transplantation 279
- Induction of neoplasia by ^{140}Ba in mice 293
- ^{32}P pyrophosphate in the treatment of persistent metastatic bone pain 327
- Induction of pituitary tumours by combination of oestrogenic hormones and ^{90}Sr 373
- Nuclear imaging of pulmonary metastases in thyroid carcinoma 401
- Hypothyroidism following ^{131}I therapy for hyperthyroidism in relation to immunologic parameters 449
- Thyroid treatment and its possible influence on occurrence of malignant tumors after diagnostic ^{131}I 455
- Radiation physics**
- Laryngeal carcinoma—IV—Analysis of treatment results using the Cohen model 3
- Simple experimentally derived algorithm for computer calculated dose rates associated with ^{13}Cs gynecologic insertions 37
- Procedures in external radiation therapy dosimetry with electron and photon beams with maximum energies between 1 and 50 MeV 55
- Effect of different radiation fractionation schedules on metastases from an oesophageal carcinoma 99
- Depth dose data for 4 MeV linear accelerators with lead or uranium field flatteners 107
- Dual photon absorptiometry in lumbar vertebrae 111
- Microdosimetry—I—Use of secondary electron emission 115
- Quantum fluctuations in roentgen imaging 129
- Collimation of high energy electron beams 199
- Microdosimetry—II—Secondary electron emission to simulate two target models 209
- Dose distribution around radium arrays used in the treatment of uterine carcinoma 229
- Electron and photon beams from a 50 MeV racetrack microtron 305
- Microprocessor system for tracking isodensity lines in film dosimetry 405
- Photon energy spectra of a heavily filtered 300 kV therapy unit 411
- Technique**
- Computation of dose distributions for radioactive seed implants 41
- Radiation biology**
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice—I—Transplanted tumour series 17
- Effect of syngeneic bone marrow and thymus cell transplantation to ^{90}Sr irradiated mice 29
- Effects of washing on phytohemagglutinin responsiveness of lymphocytes from irradiated patients 45
- Pentagastrin calcium and whisky stimulated serum calcitonin in medullary carcinoma of the thyroid 85
- Natural killer activity in peripheral lymphocyte population following radiation therapy 139
- Effect of different ^{90}Sr doses on the microscopic structure of foetal mouse ovaries 145
- Chromosome counts of ^{90}Sr injected osteosarcomas in mice—II—Slow and fast growing tumours in hyper and non hyperimmunized hosts 153
- Chloramphenicol toxicity in radiation disease 193
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice—III—In vitro transplanted tumours in vitro cultures and retransplanted cultured cells 215
- Age and dose related carcinogenicity of ^{90}Sr 223
- Sensitivity of cells in exponential and stationary growth phase to combined treatment with radiation and Quinacrine 245
- Effect of single dose irradiation on the proliferation kinetics in a human malignant melanoma in athymic nude mice 261
- Chromosome counts of ^{90}Sr induced osteosarcomas in mice—IV—When using tumours of predetermined age for transplantation 279
- Radiation sensitivity of DNA molecules in situ in normal and neoplastic tissues of mice 285
- Induction of neoplasia by ^{309}Ba in mice 293
- Influence of thyroid hormones on erythropoiesis and radiation resistance of C 57 black mice 299
- Transient intestinal ischaemia induced by degradable starch microspheres—Experiments in the cat 343
- Effects of acute gamma irradiation on spermatogenesis as revealed by flow cytometry 361
- Uptake of serotonin liberated by irradiation of rabbits and mice 369
- Induction of pituitary tumours by combination of oestrogenic hormones and ^{90}Sr 373
- Effects of proton irradiation on intra axonal transport of acetylcholine and cholinergic enzymes in rat sciatic nerve 387
- Irradiation combined with Bleomycin treatment of synchronized cells in culture underoxic and hypoxic conditions 395
- Iron incorporation after single and fractionated irradiation of infant mice 483
- Effects of hyperthermia on a neurogenic rat cell line (BT₄C) in culture 489
- Radiation injury**
- Irradiation injury of bone tissue—A vital microscopic method 235
- Dose effect relationships in radiation myelopathies 271

LIST OF AUTHORS

- Albrektsson T 235
 Alert J 13 481
- Baral E 139
 Barton R 425
 Beldarraun L 13 481
 Benes L 193
 Bergman H 17 153 215 279
 Bierke F 29 223 293 373
 Bjarngard B E 411
 Bjorkholm E 161 241
 Blomgren H 139
 Booy S 387
 Brahma A 199 305
 Broome Karlsson A 29 223 293 373
 Brustad T 761
 Burlin T E 115 209
- Calhoun D L 4 1
 Cao S 433
 Chang C 433
 Chang Y 433
 Chauvergne J 183
 Chen T S 411
- Dahl O 489
 Dahlstrom A 387
 Dawalibi H 37
 Debruyne G 169
 De Schryver A 169
 Durand M 183
- Edsmyr H 139 395
 Eghbali H 183
 El Mahdi A M 335
 Emmertsen K 85
 von Eyben F 81
- Felling M 343
 Forsberg B J 115 209
- Gadeberg C Ch 331
 de Gans J 405
 Geraci J P 91
 Gerber G B 483
 Godhe W 361
- Griffin T W 439
 Groudmie M T 439
- Hacker U 361
 Hakama M 255
 Hakansson C H 99
 Hallsten L 321
 Hammar E 99
 Hansson T H 111
 Haraldsson I 373
 Hellekant C H 321
 Hjelm Hansen M 3
 Hørn B 183
 Hørn Simon G 183
 Holdorff B 271
 Holm L E 449 455
 Holten I 401
 Horuchi J 45
 Hosek B 299
 Hvid Hansen H 85
- Isacson C 327
- Jacobsson M 235
 Jimenez J 13 481
 Johnson J 439
 Jonsson K 81 321
 Jorgensen K 357
 Jose B 421
 Jose L 425
- Kase K R 411
 Kelsey C A 41
 Kraepelin T 305
 Kusk H 425
 Kurtz J M 439
- van der Laarse R 405
 Lagarde C 183
 Lamm J L 99
 Landberg T 99
 Lane R G 41
 Laramore G E 439
 Larsson P A 387
 Lax I 199
 Lekven J 343
 Lemstra W 369
- Lindholm C 417
 Lindmo T 261
 Lattbrand B 245 395 417
 Liu T 433
 Ljungquist U 81
 Lofroth P O 417
 Lonnbeck C 327
 Lote K 343
 Lundell G 327 449
- MacDonald J C F 229
 Maes J 483
 Maree D 183
 de Mascarel A 183
 Matsubara S 45
 Mattsson W 81 321
 Mercke C 99
 Midander J 245 395
 Mikeska J 299
 Mohammed A 421
 Montalvo J 13 481
 Mosekilde L 85
- Nair R 107
 Neuková J 193 299
 Nielsen H E 85 331
 Nilsson A 17 29 223 293 373
 Nilsson P 99
- Okada S 285
 Ono T 285
- Parker R G 439
 Peebles W J 335
 Petrun B 139
 Petrovich Z 425
 Pirtoli L 353
 Pospisil M 193 299
- Rasmusson B 461
 Rice D 425
 Richard P 183
 Roca C 13 481
 Rofstad E K 261
 Romback C 145
- Roos B D 111
 Rosander K 387
 Rosen I 1 41
 Rosengren B 343 387
 Russell A H 439
- Sakamoto K 285
 Santoni R 353
 Sasaki M S 45
 Schlichting J 357
 Schumann J 361
 Sharma P D 279
 Shibuya H 45
 Silfversward C 161 241
 Skoldborn H 111
 Soderborg B 377
 Soini J 255
 Son Y H 177
 Stendahl U 467
 Stender L E 139
 Strid K 129
 Svanes K 343
 Svensson H 305
- Taanung E 401
 Tkadlecek L 193
 Tobin D A 421
 Tong D 439
 Turalba C I C 335
 Turesson I 235
- Vácha J 193
 VandeKerckhove D 169
 Velcovsky V 193
 Veninga T 369
 Vuklicka S 193
- Wahlby L 251
 Walinder G 273
 Wasserman J 139
 Werner B 327
 Westman G 251
 Willén H 467
 Willén R 467
 Worm A M 401
 Wrede D E 37 107

LIST OF SUPPLEMENTS TO ACTA RADIOLOGICA

Nos 201-363

(Issued 1980)

For Suppl. Nos 1-200 inclusive see list issued 1979 in Vol 20 Fasc 6

The supplements are published from time to time and are not included in the subscription rate. Prices and year of publication of numbers already issued are detailed below

- 201 GIOVANNI DI CHIRO RISA encephalography and conventional neuroradiologic methods. A comparative study 1961 Price Sw Kr 35
- 202 LARS BJÖRN Velopharyngeal function in connected speech. Studies using tomography and cineradiography synchronized with speech spectrography 1961 Price Sw Kr 25
- 203 BENGT O NYLÉN Cleft palate and speech. A surgical study including observations on velopharyngeal closure during connected speech using synchronized cineradiography and sound spectrography 1961 Price Sw Kr 75
- 204 S E KJELLBERG B NORDENSTRÖM U RLDHE V O BJÖRN and G MALMSTRÖM Cardioangiographic studies of the mitral and aortic valves 1961 Price Sw Kr 30
- 205 GUNNAR CARLBERGER Kinetics and distribution of radioactive cobalt administered to the mammalian body 1961 Price Sw Kr 30
- 206 HANS MOELL Kidney size and its deviation from normal in acute renal failure. A roentgendagnostic study 1961 Price Sw Kr 25
- 207 LEIF KULD HANSEN Micturition cystourethrography with automatic serial exposures. An opinion on the value of the method 1961 Price Sw Kr 30
- 208 FINN LUNDWALL Cancer of the vulva. A clinical review 1961 Price Sw Kr 30
- 209 ILIARI LINDGREN Anatomical and roentgenologic studies of tuberculous infections in BCG vaccinated and non vaccinated subjects with biophysical investigations of calcified foci 1961 Price Sw Kr 25
- 210 PER ERIN E BERGNER The significance of certain tracer kinetical methods especially with respect to the tracer dynamic definition of metabolic turnover 1962 Price Sw Kr 30
- 211 P VLORINEN P ANTILA U WEGELIUS A KAUPPIA and E KOIVISTO Renal cortical index and other roentgenographic renal measurements 1962 Price Sw Kr 25
- 212 LARS ANDRÉN Pelvic instability in newborns with special reference to congenital dislocation of the hip and hormonal factors. A roentgenologic study 1962 Price Sw Kr 30
- 213 NILS-MAGNUS ÖHLSSON Left heart and aortic blood flow in the dog. Precision motion analysis of high speed (270 frames/sec) cinefluorographic recordings 1962 Price Sw Kr 35
- 214 BENGT TIERNBERG Lymphography. An animal study on the diagnosis of χ^2 carcinoma and inflammation 1962 Price Sw Kr 35
- 215 PAAVO KILANI Periarthrosis calcarea of the shoulder joint. Its differentiation from other stiff and painful shoulders 1962 Price Sw Kr 30
- 216 P EDHOLM I FERNSTRÖM K LINDBLOM and E I SELDINGER Roentgen television in practice with special regard to puncture examinations 1962 Price Sw Kr 35
- 217 FOLKE EDSMYR Carcinoma of the vulva. An analysis of 560 patients with histologically verified squamous cell carcinoma 1962 Price Sw Kr 30
- 218 P SOILA M GYÖRÖS O KALPPILA and L PIRKONEN Wasserlösliche viskosierte wasserlösliche und jodolige Kontrastmittel in der Hysterosalpingographie. Vergleichende Untersuchungen 1962 Price Sw Kr 25
- 219 STIG SANDHARA Hiatal incompetence. Studies on mechanics and principles of examination for hiatus hernia and gastro-oesophageal reflux 1963 Price Sw Kr 25
- 220 MAX LUNDBERG Free movements in the temporomandibular joint. A cineradiographic study 1963 Price Sw Kr 30
- 221 ÅKE NORHAGEN Selective angiography of the hepatic veins. Experimental investigations of basal circulatory dynamics 1963 Price Sw Kr 35
- 222 ERLING HAMMER-JACOBSEN Genetically significant radiation doses in diagnostic radiology 1963 Price Sw Kr 35
- 223 ASTRID BROHULT Alkylglycerols and their use in radiation treatment. An experimental and clinical study 1963 Price Sw Kr 30
- 224 CARL-OLOF ÖVENFORS Pulmonary interstitial emphysema—An experimental roentgendagnostic study 1964 Price Sw Kr 35
- 225 GEORG THEANDER Variation in shape of gallbladder during cholecystography 1964 Price Sw Kr 30
- 226 HILGO BOGREN The composition and structure of human gallstones 1964 Price Sw Kr 30
- 227 LARS NORDQVIST The sagittal diameter of the spinal cord and subarachnoid space in different age groups—A roentgenographic post mortem study 1964 Price Sw Kr 30

- 228 LENNART VICTORIN Bone resorption in cases with complete upper denture — A quantitative roentgenographic photogrammetric study 1964 *Price Sw Kr 30*
- 229 ARNFINN ENGESET Irradiation of lymph nodes and vessels—Experiments in rats with reference to cancer therapy 1964 *Price Sw Kr 30*
- 230 LARS HOLLENDER Determining the elements of the interior orientation in roentgenography 1964 *Price Sw Kr 30*
- 231 HANS HENRIK HOLM The hydrodynamics of micturition—Examination by means of micro manometer and uroflowmeter of the hydrodynamic conditions in normal subjects and in patients suffering from obstruction in the posterior part of the urethra 1964 *Price Sw Kr 30*
- 232 EBBE CEDERQUIST Clinical application of whole body counting of ^{87}Sr and ^{45}Ca in patients with and without widespread malignant skeletal disease 1964 *Price Sw Kr 30*
- 233 SVEN PAULIN Coronary angiography—A technical anatomic and clinical study 1964 *Price Sw Kr 40*
- 234 TROELS MUNKNER The influence of para-aminosalicylic acid on the I^{131} metabolism 1965 *Price Sw Kr 30*
- 235 ANDERS LUNDERQUIST Angiography in carcinoma of the pancreas 1965 *Price Sw Kr 35*
- 236 RUNE WALSTAM Studies on therapeutic short distance and intracavitary gamma beam techniques—Physical considerations with special reference to radiation protection 1965 (Out of print)
- 237 KAI SETÄLÄ Differences in pharmacodynamic response to colchicine between benign and malignant epidermal hyperplasias—An experimental study in skin tumor resistant mice 1965 *Price Sw Kr 30*
- 238 UO ERIKSON Circulation in traumatic amputation stumps—An angiographical and physiological investigation 1965 *Price Sw Kr 35*
- 239 CARL GUSTAF STANDERTSKJÖLD-NORDENSTAM The pulmonary circulation during pneumonia—A cine densigraphic study 1965 *Price Sw Kr 35*
- 240 ANTTI CEDERBERG Granulocyte distribution in bone marrow, blood and different organs in whole body irradiated rats 1965 *Price Sw Kr 35*
- 241 KAI SETÄLÄ Decorporation of radiostrontium Ra radioactive assay techniques—An experimental study on mice 1965 *Price Sw Kr 30*
- 242 SHINJI TAKAHASHI Conformation radiotherapy—Rotation techniques as applied in radiography and radiotherapy of cancer 1965 *Price Sw Kr 40*
- 243 J. TH. VAN DER WERFF Radioactive bismuth ^{209}Bi —Experimental studies and clinical applications 1965 *Price Sw Kr 35*
- 244 SAMUEL S. KUROHARA Effects of ionizing radiation on creatine metabolism in patients treated for malignancy and in rats 1965 *Price Sw Kr 35*
- 245 PER WESTLING Studies of the prognosis in Hodgkin's disease 1965 *Price Sw Kr 35*
- 246 SVEN GOTTMAR ERICSSON Quantitative microradiography of cementum and abraded dentine—A methodological and biological study 1965 *Price Sw Kr 35*
- 247 MAURI WILJASALO Lymphographic differential diagnosis of neoplastic disease 1965 *Price Sw Kr 35*
- 248 SVEN SCHELLER Roentgenographic studies on ossification of the distal femoral epiphysis 1965 *Price Sw Kr 30*
- 249 ROAR NISSEN MEYER Castration as part of the primary treatment for operable female breast cancer—statistical evaluation of clinical results 1965 *Price Sw Kr 35*
- 250 ELIS BERVEN SVEN HULTBERG HANS L. KOTTMEIER ROLF SIEVERT LARS SANTESSON BENGT SYLVEN The first fifty years Radiumhemmet 1910-1937 and King Gustaf V Jubilee Clinic 1938-1965 *Price Sw Kr 30*
- 251 MATS HAVERLING Renal phlebography—An experimental study in the pig 1966 *Price Sw Kr 30*
- 252 GUNNAR WESTBERG Gas myelography and percutaneous puncture in the diagnosis of spinal cord cysts 1966 *Price Sw Kr 30*
- 253 SVEN IVAR SELDINGER Percutaneous transhepatic cholangiography 1966 *Price Sw Kr 35*
- 254 FIRST NORDIC RADIATION PROTECTION CONFERENCE Proceedings Stockholm 1966 Edited by K. Lidén and Erik Lindgren *Price Sw Kr 35*
- 255 LAWRENCE JOSEPH VAN CURA Application of diodes in computers in radiation dosimetry 1966 *Price Sw Kr 35*
- 256 HANS LUDIN Aortography Fluid dynamics technical problems 1966 *Price Sw Kr 35*
- 257 HJALMAR BOLIN Contrast medium in kidney disease—angiography—A densitometric method for estimation of renal function 1966 *Price Sw Kr 30*
- 258 ELISABETH JOHANNISSON PER KOLSTAD GUNNAR SÖDERBERG Cytologic vascular and histologic patterns of dysplasia carcinoma in situ and early invasive carcinoma of the cervix 1966 *Price Sw Kr 30*
- 259 PAUL EDHOLM Anatomical angles determined from two radiographic projections—Instrument description and measurement technique 1966 *Price Sw Kr 40*
- 260 TORSTEN ALMÉN A steering device for selected angiography and some vascular and enzymatic reactions observed in its clinical application 1966 *Price Sw Kr 40*
- 261 KAI SETÄLÄ BJÖRN LINDROOS and OTTO NYSSÖNEN Cancer chemotherapy studies cytoplasmic barbiturate in malignant epidermal cells against the effect of colchicine—An electron microscopic study in mice 1967 *Price Sw Kr 25*
- 262 KLAS ROSENGREN Hyaline membrane disease—radiological investigation in rabbits 1967 *Price Sw Kr 35*
- 263 JAN NILLSON Angiography in tumours of the urinary bladder 1967 *Price Sw Kr 35*
- 264 PER ERIK HEIKEL Postmortal changes of the lungs—A roentgenographic microscopic and bacteriological follow up study on a pediatric series and on animals with experimental pneumonia 1967 *Price Sw Kr 30*
- 265 KAI SETÄLÄ OTTO NYSSÖNEN and BJÖRN LINDROOS Ultrastructural changes in benign and malignant epidermal states in mice after topical beta radiation 1967 *Price Sw Kr 30*

- '66 GÖRAN NYLANDER Vascular response to vasopressin as reflected in angiography—An experimental study in the dog 1967 *Price Sw Kr 35*
- '67 JOHAN FOLIN Angiography in renal tumours—Its value in diagnosis and differential diagnosis as a complement to conventional methods 1967 *Price Sw Kr 35*
- '68 EERO TALA Carcinoma of the lung—A retrospective study with special reference to pre diagnosis period and roentgenographic signs 1967 *Price Sw Kr 35*
- '69 CARL O HENRIKSSON Iodine 125 as a radiation source for odontological roentgenology 1967 *Price Sw Kr 35*
- '70 CATIONS IN INTRAVASCULAR CONTRAST MEDIA AND DEVELOPMENT OF SPECIFIC METRIZOATE FORMULAS — PHARMACOLOGIC AND CLINICAL STUDIES *Proc Sympo sia at Copenhagen November 1964 and Sandefjord September 1966* 1967 *Price Sw Kr 40*
- '71 ERNA TARKIÄINEN Intracostal vein meningorachidography—A technical anatomic and clinical study 1967 *Price Sw Kr 35*
- '72 ALLAN LUNDERQUIST Arterial segmental supply of the liver—An angiographic study 1967 *Price Sw Kr 35*
- '73 KAI SETÄLÄ MAX SINRALA OTTO NYSSÖNEN and ERNA TARKIÄINEN Quantitative three dimensional scintillography of the stomach with technetium (^{99m}Tc) 1967 *Price Sw Kr 30*
- '74 PER BERGSSÖ Radiation induced early changes in size and vascularity of cervical carcinoma—A colposcopic and clinical study 1968 *Price Sw Kr 35*
- '75 SUNE ERICSSON The parotid gland in subjects with and without rheumatoid arthritis 1968 *Price Sw Kr 40*
- '76 ROLF JENSEN Anterior teeth relationship and speech—Studies using cineradiography synchronized with speech recording 1968 *Price Sw Kr 35*
- '77 SVEN AHLBACK Osteoarthritis of the knee—A radiographic investigation 1968 *Price Sw Kr 35*
- '78 IRÉNE SJÖGREN KJELL BERGSTRÖM and HERMAN LODIN Echoencephalography in infants and children. Comparison with cerebral pneumography in measuring ventricular size 1968 *Price Sw Kr 35*
- '79 BERTIL JÄRPLID Radiation induced asymmetry and lymphoma of thymus in mice 1968 *Price Sw Kr 35*
- '80 ERKKI M. LAASONEN Information transmission in roentgen diagnostic chains—Experimental and clinical studies 1968 *Price Sw Kr 35*
- '81 RASMUS STENSTRÖM Arthrography of the knee joint in children—Roentgenologic anatomy diagnosis and the use of multiple discriminant analysis 1968 *Price Sw Kr 35*
- '82 KARL KARLSTEDT Carcinoma of the uterine corpus—Factors bearing on the curability 1968 *Price Sw Kr 35*
- '83 LEO STJERNVALL Pharmacodynamic response of epidermal hyperplasias to topical vinblastine treatment 1968 *Price Sw Kr 35*
- '84 HANS FLOD Distribution and kinetics of labelled vitamin B₁₂ 1968 *Price Sw Kr 35*
- '85 ERKKI KOIVISTO Comparative study of roentgen diagnostic classifications—Computer analysis of 124496 roentgen reports 1969 *Price Sw Kr 35*
- '86 JÖRGEN JENSEN Malformations of the inner ear in deaf children—A tomographic and clinical study 1969 *Price Sw Kr 35*
- '87 PENTTI J. TASKINEN Radiotherapy and TNM classification of cancer of the larynx—A study based on 1447 cases seen at the Radiotherapy Clinic of Helsinki during 1936–1961 1969 *Price Sw Kr 35*
- '88 ROBERT T. NASH Decision processes employing radioisotope scanning 1969 *Price Sw Kr 35*
- '89 SIRKKA WILJASALO Lymphographic polymorphism in Hodgkin's disease—Correlation of lymphography to histology and duration 1969 *Price Sw Kr 35*
- '90 ULF WELANDER Multicolor combination images in subtraction angiography—A new photographic method and its applications 1969 *Price Sw Kr 40*
- '91 ILONA SCHRECK PUROLA Failure of malignant epidermal cells to respond to vinblastine sulfate—A study in skin tumor resistant mice 1969 *Price Sw Kr 35*
- '92 GIOVANNI RUGGIERO GIANFRANCO CRISTI and CLAUDIO TREVISAN Clinical aspects of encephalography 1969 *Price Sw Kr 30*
- '93 PEKKA VIRTAMA and TAPPO HELELA Radiographic measurements of cortical bone—Variations in a normal population between 1 and 90 years of age 1969 *Price Sw Kr 20*
- '94 L. STJERNVALL E. E. NISKANEN and J. TARKANEN Penetration of cytoplasmic barrier in malignant epidermal hyperplasia by colchicine in dimethyl sulfoxide—A polarization microscopic study in skin tumor resistant mice 1969 *Price Sw Kr 20*
- '95 KAARINA TOURU KAISILA Heart size determination by photofluorography 1970 *Price Sw Kr 35*
- '96 HANS ROVINGS OTOSCLEROSIS—A tomographic clinical study 1970 *Price Sw Kr 35*
- '97 PER LANGE LAND Population screening for female breast tumours A clinical investigation 1970 *Price Sw Kr 35*
- '98 JOHAN EUGEN Effect of cysteine on chromosome aberrations induced by radiation of human lymphocytes in vitro 1970 *Price Sw Kr 30*
- '99 RUNE SUNDGREN Selective angiography of the left gastric artery 1970 *Price Sw Kr 35*
- '00 NIELS KRØDGAARD The lower urinary tract in infancy and childhood—Micturition cinematography with simultaneous pressure flow measurement 1970 (Out of print)
- '01 M. VIKKARI Ultrasound examination of pleural plaques—Experimental pathologic and clinical studies 1970 *Price Sw Kr 35*
- '02 INGEMAR JOELSSON Radiotherapy of carcinoma of the uterine cervix with special regard to external irradiation 1970 *Price Sw Kr 35*
- '03 KAARINA AANTTA Location of the placenta — A comparison between radiography ultrasound thermography isotopes 1971 *Price Sw Kr 25*
- '04 LENNART DIENER Intraosseous phlebography of the lower limb—Postmortem investigation of thrombotic venous disease 1971 *Price Sw Kr 40*
- '05 BERNDT STRÖMBERG The normal and diseased superficial flexor tendon in race horses—A morphologic and physiologic investigation 1971 *Price Sw Kr 35*

- 306 TRYGVE AAKHUS Angiography in acute mechanical obstruction of the small intestine 1971 *Price Sw Kr 40*
- 307 PERTTU METSALA Effect of dimethyl sulfoxide (DMSO) on cytoplasmic barrier of malignant epidermal cells—An investigation in skin tumor resistant mice 1971 *Price Sw Kr 35*
- 308 JØRGEN RYGÅRD Mechanism of blood clearance of colloidal gold in mice—An atoxic clinical investigation using activation analysis 1971 *Price Sw Kr 35*
- 309 LAURI PATOMAKI A mathematical model for radiation fields of telecobalt treatment units—With special reference to the isodoses of Rocus 1971 *Price Sw Kr 35*
- 310 RADIOBIOLOGIC INVESTIGATIONS Edited by Erik Lindgren and Bernhard Tribukait 1971 *Price Sw Kr 45*
- 311 HALVOR VERMUND Enhancement of radiation effects by chemotherapy 1971 *Price Sw Kr 35*
- 312 PERTTI KASKI Osteomedullography of the tibia 1971 *Price Sw Kr 40*
- 313 PROCEEDINGS OF THE SIXTH CONFERENCE OF THE NORDIC ASSOCIATION OF CLINICAL PHYSICS held in Århus Denmark 1970 Edited by C B Madsen and K Liden 1972 *Price Sw Kr 45*
- 314 BIRGER HELIN Heart volume in human kidney transplantation 1972 *Price Sw Kr 25*
- 315 UNO WERLILUS Angiography of the hand Clinical and postmortem investigations 1972 *Price Sw Kr 35*
- 316 P E S PALMER Haemangiosarcoma of Kaposi 1972 *Price Sw Kr 35*
- 317 JHANI RAUSTE Lymphographic findings in granulomatous inflammations and connective tissue diseases—Differential diagnosis between these diseases and lymphomas 1972 *Price Sw Kr 30*
- 318 OVI MATSSON Formation of the tomographic image—With special reference to blurring 1972 *Price Sw Kr 35*
- 319 PROGRESS IN VETERINARY RADIOLOGY Proceedings of the 2nd International Conference of Veterinary Radiologists held in Stockholm 1970 Edited by Sten Erik Olsson 1972 *Price Sw Kr 45*
- 320 TJAKKO KUIPERS Carcinoma of the uterine cervix Aspects of clinical oncology in patients referred for radiation therapy 1972 *Price Sw Kr 50*
- 321 BO LUNDSTRÖM Angiographic abnormalities following percutaneous needle biopsy of the kidney 1972 *Price Sw Kr 40*
- 322 LARS BLONQUIST Mode of accumulation of iodo phenylalanines in the exocrine pancreas and certain tumours 1972 *Price Sw Kr 40*
- 323 INGER BROLIN Radiologic reporting 1973 *Price Sw Kr 40*
- 324 TIMO TELARANTA The role of host tissue in skin carcinogenesis—An investigation with skin tumor resistant and skin tumor susceptible mice 1973 *Price Sw Kr 35*
- 325 NILS GUNNAR LINDQUIST Accumulation of drugs on melanin 1973 *Price Sw Kr 40*
- 326 JOHN ERIK JOHNSON Hystorography and diagnostic curettage in carcinoma of the uterine body 1973 *Price Sw Kr 40*
- 327 ERIC BERGQUIST Tentorial notch and adjacent major vessels in carotid angiography 1973 *Price Sw Kr 45*
- 328 O HASSLER and S O HIETALA Angiographic abnormalities in the urinary bladder wall after irradiation Part I Animal experiments Part II Clinical investigation 1973 *Price Sw Kr 45*
- 329 OLOF ECKERDAL Tomography of the temporomandibular joint—Correlation between tomographic image and histologic sections in a three dimensional system 1973 *Price Sw Kr 40*
- 330 JORMA RANTANEN Radiation injury of connective tissue—A biochemical investigation with experimental granuloma 1973 *Price Sw Kr 40*
- 331 FRANZ PAUL PROBST Congenital defects of the corpus callosum—Morphology and encephalographic appearances 1973 *Price Sw Kr 50*
- 332 GUDRUN ALM CARLSSON Dosimetry at interfaces—Theoretical analysis and measurements by means of thermoluminescent LiF 1973 *Price Sw Kr 40*
- 333 MATTI VALLE Postoperative coronary angiography 1973 *Price Sw Kr 40*
- 334 I JOELSSON A SANDRI and H L KOTTMEIER Carcinoma of the uterine corpus—A retrospective survey of individualized therapy 1973 *Price Sw Kr 40*
- 335 METRIZAMIDE A NON IONIC WATER SOLUBLE CONTRAST MEDIUM—Experimental and preliminary clinical investigations 1973 *Price Sw Kr 50*
- 336 SVEN SCHELLER and LARS MÅRTENSON Traumatic dislocation of the patella A radiographic investigation 1974 *Price Sw Kr 50*
- 337 OSSU KORHOLA Myocardial scintigraphy and estimation of regional blood flow with xenon 133 1974 *Price Sw Kr 40*
- 338 KURT ÅSTRAND and SVEN REICHMANN Optimised tomography Theoretical and practical analyses of the elimination of depiction errors in tomography 1974 *Price Sw Kr 40*
- 339 ILKKA SURAMO Lymphography in tuberculosis 1974 *Price Sw Kr 40*
- 340 EEEVA NORDMAN ⁷⁵Se sodium selenite scintigraphy in diagnosis of tumours 1974 *Price Sw Kr 45*
- 341 ILPO LAUTEALA Pelvimetry with image intensifier camera A low radiation dose method 1974 *Price Sw Kr 50*
- 342 ANDERS MØLLER Pneumography in paraventricular and intraventricular tumours of the posterior fossa 1974 *Price Sw Kr 60*
- 343 HÅKAN JORULF Roentgen diagnosis of intraperitoneal fluid A physical anatomic and clinical investigation 1975 *Price Sw Kr 55*
- 344 Skeletal development growth rate and hip dysplasia Experimental investigations with special reference to the effect of estrogens growth hormone and nutrition Edited by Sten Erik Olsson 1975 *Price Sw Kr 70*
- 345 HANS KUISS and FAIZ M KHAN Nominal standard dose and tumor standard dose Tables for radiation therapy planning and analysis 1975 *Price Sw Kr 65*
- 346 Computer tomography of brain lesions Edited by Erik Lindgren 1975 *Price Sw Kr 73*
- 347 Tenth Symposium Neuroradiologicum Edited by Erik Lindgren 1975 *Price Sw Kr 148*

- 348 SEPPO LAHDE Cineangiographic determination of left ventricular volume—Accuracy of methods 1976 *Price Sw Kr 50*
- 349 KAJ TALLROTH Lymphatic dissemination of bone and soft tissue sarcomas—A lymphographic investigation 1976 *Price Sw Kr 50*
- 350 BO FREDRIK ZACHRISSON Thyroid angiography 1976 *Price Sw Kr 65*
- 351 KARL GUSTAV STRID Analysis of secondary screening with special reference to grids for abdominal radiography 1976 *Price Sw Kr 65*
- 352 SEPPO SAKSANEN Relationship between encephalographic measurements and social performance—A statistical analysis of 915 patients with partial or permanent occupational disability 1976 *Price Sw Kr 60*
- 353 T R MÖLLER U B NORDBERG T GUSTAFSSON J E JOHNSON T G LANDBERG and G SVAHN TAPFER Planning control and documentation of external beam therapy 1976 *Price Sw Kr 75*
- 354 PAULI HEKALI Coronary angiography and clinical symptomatology 1976 *Price Sw Kr 65*
- 355 Metrizamide Amipaque The non ionic water soluble contrast medium Further clinical experience in neuroradiology Edited by Erik Lindgren 1977 *Price Sw Kr 150*
- 356 HANS DAHLIN ULF WELANDER and HERMANN WILBRAND Clinical comparison between monochrome colour film and black and white film 1978 *Price Sw Kr 70*
- 357 NILS G BJURSTAM Radiography of the female breast and axilla 1978 *Price Sw Kr 105*
- 358 Osteochondrosis in domestic animals—I—Pathogenesis and pathology in pigs horses bulls turkeys and broilers with comparative aspects on osteochondritis dissecans in man Edited by Sten Erik Olsson 1978 *Price Sw Kr 150*
- 359 LEIF I SAMUELSSON Mechanism for exoelectron emission mainly from LiF 1979 *Price Sw Kr 70*
- 360 PER SPANNE Thermoluminescence dosimetry in the μGy range—Theoretical and experimental investigations of the optimum performance of a LiF TLD system 1979 *Price Sw Kr 75*
- 361 ÅKE GULLMO Herniography—The diagnosis of hernia in the groin and incompetence of the pouch of Douglas and pelvic floor 1980 *Price Sw Kr 100*
- 362 Iohevol A non ionic contrast medium Pharmacology and toxicology Edited by Erik Lindgren 1980 *Price Sw Kr 120*
- 363 STIG A LARSSON Gamma camera emission tomography—Development and properties of a multi sectional emission computed tomography system 1980 *Price Sw Kr 100*

- 306 TRYGVE AAKHUS Angiography in acute mechanical obstruction of the small intestine 1971 *Price Sw Kr 40*
- 307 PERTTU MITSALA Effect of dimethyl sulfoxide (DMSO) on cytoplasmic barrier of malignant epidermal cells—An investigation in skin tumor resistant mice 1971 *Price Sw Kr 35*
- 308 JØRGEN RYGDÅRD Mechanism of blood clearance of colloidal gold in mice—An atoxic clinical investigation using activation analysis 1971 *Price Sw Kr 35*
- 309 LAURI PATOMAKI A mathematical model for radiation fields of telecobalt treatment units—With special reference to the isodoses of Rocus 1971 *Price Sw Kr 35*
- 310 RADIOBIOLOGIC INVESTIGATIONS Edited by Erik Lindgren and Bernhard Tribukait 1971 *Price Sw Kr 45*
- 311 HALVOR VERMUND Enhancement of radiation effects by chemotherapy 1971 *Price Sw Kr 35*
- 312 PERTTI KASKI Osteomedullography of the tibia 1971 *Price Sw Kr 40*
- 313 PROCEEDINGS OF THE SIXTH CONFERENCE OF THE NORDIC ASSOCIATION OF CLINICAL PHYSICISTS held in Århus Denmark 1970 Edited by C B Madsen and K Liden 1972 *Price Sw Kr 45*
- 314 BIRGER HELIN Heart volume in human kidney transplantation 1972 *Price Sw Kr 25*
- 315 UNO WERLIGUS Angiography of the hand Clinical and postmortem investigations 1972 *Price Sw Kr 35*
- 316 P E S FAIMER Haemangiosarcoma of Kaposi 1972 *Price Sw Kr 35*
- 317 JUHANI RÄSTÉ Lymphographic findings in granulomatous inflammations and connective tissue diseases—Differential diagnosis between these diseases and lymphomas 1972 *Price Sw Kr 30*
- 318 OVE MATSSON Formation of the tomographic image—With special reference to blurring 1972 *Price Sw Kr 5*
- 319 PROCEEDINGS IN VETERINARY RADIOLOGY Proceedings of the 2nd International Conference of Veterinary Radiologists held in Stockholm 1970 Edited by Sten Erik Olsson 1972 *Price Sw Kr 45*
- 320 TIAKKO KLIPERS Carcinoma of the uterine cervix Aspects of clinical oncology in patients referred for radiation therapy 1972 *Price Sw Kr 50*
- 321 BO LUNDSTRÖM Angiographic abnormalities following percutaneous needle biopsy of the kidney 1972 *Price Sw Kr 40*
- 322 LARS BLOMQUIST Mode of accumulation of radio-phenylalanines in the exocrine pancreas and certain tumours 1972 *Price Sw Kr 40*
- 323 INGER BROLIN Radiologic reporting 1973 *Price Sw Kr 40*
- 324 TIMO TELARANTA The role of host tissue in skin carcinogenesis—An investigation with skin tumor resistant and skin tumor susceptible mice 1973 *Price Sw Kr 35*
- 325 NILS GUNNAR LINDQUIST Accumulation of drugs on melanin 1973 *Price Sw Kr 40*
- 326 JOHN ERIK JOHNSON Hysterography and diagnostic curettage in carcinoma of the uterine body 1973 *Price Sw Kr 40*
- 327 ERIC BERGQUIST Tentorial notch and adjacent major vessels in carotid angiography 1973 *Price Sw Kr 45*
- 328 O HASSLER and S O HIETALA Angiographic abnormalities in the urinary bladder wall after irradiation Part I Animal experiments Part II Clinical investigation 1973 *Price Sw Kr 45*
- 329 OLOF ECKERDAL Tomography of the temporomandibular joint—Correlation between tomographic image and histologic sections in a three dimensional system 1973 *Price Sw Kr 40*
- 330 JORMA RANTANEN Radiation injury of connective tissue—A biochemical investigation with experimental granuloma 1973 *Price Sw Kr 40*
- 331 FRANZ PAUL PROBST Congenital defects of the corpus callosum—Morphology and encephalographic appearances 1973 *Price Sw Kr 50*
- 332 GUDRUN ALM CARLSSON Dosimetry at interfaces—Theoretical analysis and measurements by means of thermoluminescent LiF 1973 *Price Sw Kr 40*
- 333 MATTI VALLE Postoperative coronary angiography 1973 *Price Sw Kr 40*
- 334 I JOELSSON A SANDRI and H L KOTTMFIER Carcinoma of the uterine corpus—A retrospective survey of individualized therapy 1973 *Price Sw Kr 40*
- 335 METRIZAMIDE A NON IONIC WATER SOLUBLE CONTRAST MEDIUM—Experimental and preliminary clinical investigations 1973 *Price Sw Kr 50*
- 336 SVEN SCHELLER and LARS MARTENSON Traumatic dislocation of the patella A radiographic investigation 1974 *Price Sw Kr 50*
- 337 OSSI KORHOLA Myocardial scintigraphy and estimation of regional blood flow with xenon 133 1974 *Price Sw Kr 40*
- 338 KURT ÅSTRAND and SVEN REICHMANN Optimised tomography Theoretical and practical analyses of the elimination of depiction errors in tomography 1974 *Price Sw Kr 40*
- 339 ILKKA SURAMO Lymphography in tuberculosis 1974 *Price Sw Kr 40*
- 340 EVA NORDMAN ⁷⁵Se sodium selenite scintigraphy in diagnosis of tumours 1974 *Price Sw Kr 45*
- 341 ILPO LAUTEALA Pelvimetry with image intensifier camera A low radiation dose method 1974 *Price Sw Kr 50*
- 342 ANDERS MÖLLER Pneumography in paraventricular and intraventricular tumours of the posterior fossa 1974 *Price Sw Kr 60*
- 343 HÅKAN JORULF Roentgen diagnosis of intraperitoneal fluid A physical anatomic and clinical investigation 1975 *Price Sw Kr 55*
- 344 Skeletal development growth rate and hip dysplasia Experimental investigations with special reference to the effect of estrogens growth hormone and nutrition Edited by Sten Erik Olsson 1975 *Price Sw Kr 70*
- 345 HANS KUISS and FAIZ M KHAN Nominal standard dose and tumor standard dose Tables for radiation therapy planning and analysis 1975 *Price Sw Kr 65*
- 346 Computer tomography of brain lesions Edited by Erik Lindgren 1975 *Price Sw Kr 73*
- 347 Tenth Symposium Neuroradiologicum Edited by Erik Lindgren 1975 *Price Sw Kr 148*

